Morganella psychrotolerans - Identification, histamine formation and importance for histamine fish poisoning

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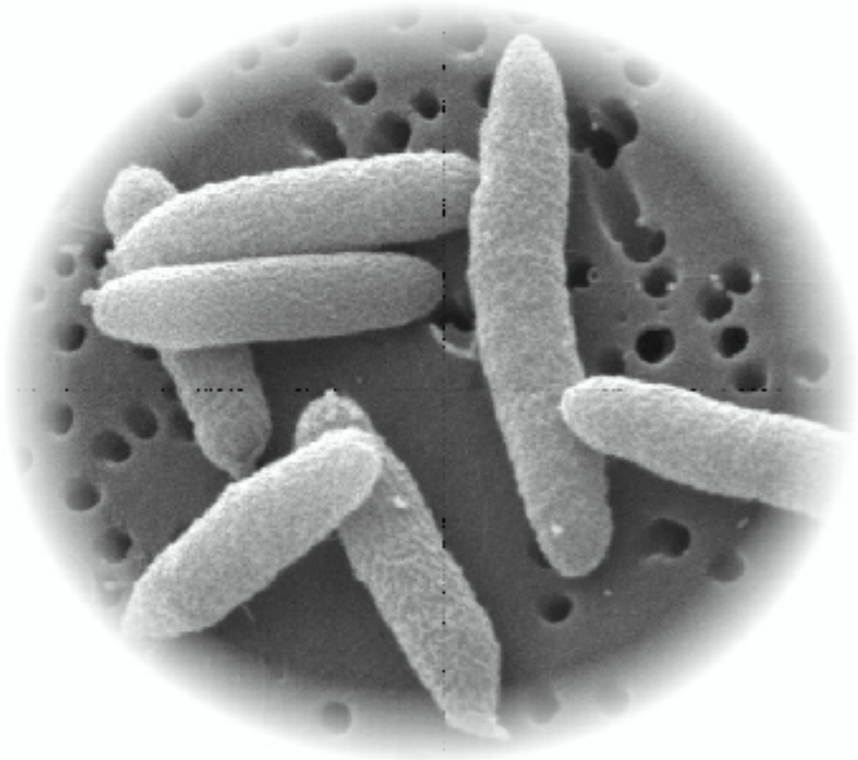
PHD THESIS

Danish Institute for Fisheries Research

Jette Emborg

Morganella psychrotolerans-
Identification, histamine foramtion and importance for histamine fish poisoning



DTU · DIFRES

Morganella psychrotolerans

Identification, histamine formation and importance for histamine fish poisoning

Ph.D. Thesis by

Jette Emborg, 2007

Department of Seafood Research,
Danish Institute for Fisheries Research,
Technical University of Denmark.

Cover illustration:

Scanning electron microscopy picture of *Morganella psychrotolerans* in 1% NaCl (30.000x magnification). Picture taken by Dagmar A. Brüggemann, Department of Food Science/Meat Science, Faculty of Life Sciences, University of Copenhagen.

Preface

The work presented in this thesis is the outcome of a Ph.D. study carried out and partial financed within the EU Integrated Project SEAFOODplus, contract no. FOOD-CT2004-506359. I was enrolled as Ph.D. student at the Technical University of Denmark (DTU). The work was carried out at

- Department of Seafood Research, Danish Institute for Fisheries Research (DIFRES), DTU, Lyngby, Denmark.
- Danish Institute for Food and Veterinary Research, Copenhagen V, Denmark (Now National Veterinary Institute at DTU).

I am deeply grateful to my supervisor Senior scientist Paw Dalgaard (DIFRES) for sharing his outstanding enthusiasm, for continuously encouraging me especially, through the difficult times, for being so generous with his time, for all his constructive advices and for his ability to see things from a different perspective. I sincerely thank my co-supervisor Senior scientist Peter Ahrens and his lab technician Kirsten Vestergaard (Danish Institute for Food and Veterinary Research) for sharing their knowledge on sequence analysis.

I wish to thank Tina Dahl Devitt for joining us and especially for her outstanding technical and independent work in the laboratory. Technician Nadereh Samieian is especially thanked for never letting me down and always being there when I needed a hand. Inge Holmberg is special gifted when it comes to troublesome HPLC equipment I wish to thank her for the assistance. Furthermore I also wish to thank my colleagues at DIFRES for being around ready for discussions, coffee and lunches.

I want to thank librarian Søren Tørper Christensen from DIFRES for his invaluable help in finding huge amounts of the most impossible literature.

Jette Emborg

July 2007

Summary

***Morganella psychrotolerans* - Identification, histamine formation and importance for histamine fish poisoning**

Histamine fish poisoning (HFP) is a worldwide problem, primarily caused by consumption of fish. Allergy-like symptoms such as flushing, rash and headache are typical symptoms of HFP. The symptoms occur within a range of few minutes to a couple of hours after ingestion of fish, containing more than 500 - 1,000 ppm histamine. Histamine seems the causative agent in the majority of the reported incidents of seafood-borne diseases in many countries. However, good statistics on the worldwide frequency of HFP are lacking since HFP is not a notifiable disease in many countries. In addition, misdiagnosis of HFP as food-allergy may occur due to the similarity in symptoms. The frequency of HFP in Denmark as well as in many other countries seems to remain unchanged even though the problem is well documented. The enzyme L-histidine decarboxylase, which is responsible for histamine formation in seafood, is produced by several species of bacteria primarily from the family of Enterobacteriaceae.

In the present thesis aspects of HFP such as symptoms, toxicology and implicated products are described. The bacterial formation of histamine and the factors affecting the formation are likewise described.

The overall purpose of the Ph.D. project was to obtain information that can assist the process of reducing the frequency of HFP related to ingestion of fresh fish and seafood products. During a three-year period, all reported incidents of HFP in Denmark were thoroughly investigated. This investigation included analysis of the symptoms observed by the patients and recorded using questionnaires, chemical characterisation of the seafood products involved, isolation of the dominating microflora and identification of the strongly histamine-producing bacteria (HPB).

The work has provided significant new information on psychrotolerant HPB and their importance for HFP. During 1955-2002, the bacteria responsible for histamine formation in relation to incidents of HFP had only been identified in five scientific publications. During the present study HPB responsible for histamine

formation were identified in five additional incidents. It was demonstrated that psychrotolerant HPB might be as important as mesophilic HPB with respect to HFP. A new species of *Morganella* was isolated and identified from seafood involded in incidents of HFP. This new species with crucial importance to seafood safety was named *Morganella psychrotolerans,* as it is capable of histamine formation at 0°C.

Control and inhibition of growth and the concomitant histamine formation by *M. psychrotolerans* and other strongly psychrotolerant (e.g. *Photobacterium phosphoreum*) or mesophilic (e.g. *Morganella morganii*) HPB might reduce the frequency of HFP. It was shown, through challengetests with inoculated tuna, that a modified atmosphere with O_2 and CO_2 inhibit the growth of *M. psychrotolerans* and *P. phosphoreum* in fresh tuna. Modified atmosphere packaging might replace the traditional vacuum packaging of fresh tuna to increase seafood safety. In cold-smoked tuna more than 5% water phase salt in combination with at shelf-life of no more than 3-4 weeks is suggested to prevent toxic concentrations of histamine by psychrotolerant HPB in the product. Finally, a model for prediction of growth and histamine formation by *M. psychrotolerans* was developed. It is the first model for histamine formation that includes both the effect of storage conditions (temperature and CO_2) and product characteristics (pH and NaCl). This model is a first step towards a quantitative assessment of consumers exposure to histamine and can be used for determination of safe shelf-life and optimisation of seafood products. The model may work as a template for similar models for other HPB. The model can be used as a decision tool by the seafood industry as well as regulatory authorities.

Sammendrag (in Danish)

***Morganella psychrotolerans* – Identifikation, histamin dannelse og betydning for histamin forgiftning**

Histamin forgiftning (HF) er et verdensomspændende problem, der primært skyldes indtagelse af fiskeprodukter. Allergilignende symptomer som rødme, udslæt og hovedpine er typiske for HF. Disse symptomer opstår fra få minutter og op til et par timer efter indtagelse af fiskeprodukter, der indeholder mere end 500-1000 ppm histamin. HF er årsag til langt de fleste rapporterede udbrud af fiskebåren sygdom i mange lande. Desværre findes der ikke opgørelser der viser hvor omfattende HF er på verdensplan, da HF i mange lande ikke skal indberettes. Desuden kan de allergilignende symptomer betyde at HF fejldiagnosticeres som fødevareallergi. Selvom HF er en velkendt forgiftning, har hyppigheden af HF i Danmark og mange andre lande ikke ændret sig væsentligt de seneste årtier. Histamin dannes ved en decarboxylering af aminosyren histidin af enzymet histidindecarboxylase. Dette enzym dannes af bakterier primært fra Enterobacteriaceae-familien.

I denne afhandling er symptomerne, toksikologien og de involverede produkter ved HF beskrevet. Desuden beskrives den bakterielle dannelse af histamin og de faktorer der påvirker histamindannelsen.

Formålet med dette ph.d. projekt har været at skabe viden der kan medvirke til en reducering i antallet af fiskebåren HF. Gennem en treårig periode blev alle rapporterede udbrud af HF i Danmark undersøgt. Ved udbrud af HF udfyldte de involverede personer et spørgeskema der omhandlende de oplevede symptomer. Eventuelle rester fra det pågældende måltid blev indsamlet og isolering af den dominerende mikroflora og identifikation af de kraftigt histaminproducerende bakterier (HPB) blev udført. Desuden blev der foretaget en kemisk karakterisering af produkter.

Projektet har skabt ny og betydningsfuld viden om kuldetolerante HPB og deres betydning for HF. I den videnskabelige litteratur fra perioden 1955-2002 har det kun været muligt at finde fem studier, omhandlende identifikation af de HB der har forårsaget HF. Det nærværende projekt har bidraget med yderligere fem. Det er

vist at kuldetolerante HPB kan være lige så betydningsfulde som mesofile HPB med hensyn til HF. En ny art af *Morganella* blev isoleret fra fersk og kold-røget tun involveret i udbrud af HF. Den nye art blev navngivet *Morganella psychrotolerans*, da den er i stand til at vokse og danne histamin ved 0°C. Identifikation af *M. psychrotolerans* kan vise sig at være vigtig i forhold til sikkerheden af fisk og fiskeprodukter.

Kontrol af vækst og histamindannelse for *M. psychrotolerans* og andre kuldetolerante bakterier (f.eks. *Photobacterium phosphoreum*) eller mesofile HB (f.eks. *Morganella morganii*) kan nedsætte hyppigheden af HF. Det er vist, at væksten og dermed histamindannelsen af både *M. psychrotolerans* og *P. phosphoreum* i fersk tun hæmmes kraftigt ved anvendelse at modificeret atmosfære pakning med O_2 og CO_2 i forhold til vakuum pakning. For at øge fødevaresikkerheden kan den traditionelle vakuum pakning erstattes med modificeret atmosfære pakning. For at hindre dannelse af histamin i toksiske koncentrationer i produkter som kold-røget tun er det foreslået, at holdbarheden ikke er længere end 3-4 uger i produkter med 5% salt i vandfasen.

I afhandlingen beskrives desuden udviklingen af en model til forudsigelse af vækst og histamindannelse af *M. psychrotolerans*. Det er den første model, der inkludere effekten af både lagringsbetingelser (temperatur og CO_2) samt produktkarakteristika (pH og salt). Modellen er det første skridt imod en kvantitativ vurdering af, hvilke mængder histamin forbrugerne udsættes for og kan benyttes som skabelon ved udvikling af tilsvarende modeller for andre HB. Produktionsvirksomheder og tilsynsmyndigheder kan f.eks. anvende modellen til fastsættelse af holdbarheder og optimering af fiskeprodukter.

Table of contents

The thesis is based on the following 5 papers:

1. **Emborg, J., Laursen, B. G. and Dalgaard, P. 2005** Significant histamine formation in tuna (*Thunnus albacares*) at 2°C - effect of vacuum- and modified atmosphere-packaging on psychrotolerant bacteria. *International Journal of Food Microbiology*, vol. 101, 263-279.

   The following parts of this publication results directly from the present Ph.D. project: (i) Investigation of an outbreak of histamine fish poisoning due to tuna. This included isolation, identification and screening for histamine production of the dominating microflora. (ii) Challengetests, where the effect of an oxygen containing modified atmosphere on histamine formation was studied as a function of the initial bacterial concentration and different profiles of storage temperatures. Part of this paper, namely storage trial 1 and 2 as well as the identification of the microbiota of yellowfin tuna at the time of processing, has previously been handed in as a part of the Master thesis: “Histamine-producing bacteria in tuna from Sri Lanka” by Birgit Groth Laursen and Jette Emborg, Danish Institute for Fisheries Research, DTU, 2003. Storage trial 3 originates from the Bachelor thesis of Jette Emborg and Birgit Groth Laursen: “Importance and formation of biogenic amines in fresh and thawed MAP fish” (in Danish), Danish Institute for Fisheries Research, DTU, 2001.

2. **Emborg, J. and Dalgaard, P. 2006** Formation of histamine and biogenic amines in cold-smoked tuna: An investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning. *Journal of Food Protection*, vol. 69, 897-906.

3. **Emborg, J., Dalgaard, P. and Ahrens, P. 2006** *Morganella psychrotolerans* sp. nov., a histamine-producing bacterium isolated from various seafoods. *International Journal of Systematic and Evolutionary Microbiology,* vol. 56, 2473-2479.

4. **Emborg, J. and Dalgaard, P. 2007** Modelling and predicting the growth and histamine formation by *Morganella psychrotolerans*. (In preparation).

5. **Dalgaard, P., Emborg, J., Kjølby, A., Sørensen, N. D., and Ballin, N. Z. 2008** "Histamine and biogenic amines - formation and importance in seafood" in *Improving Seafood Products for the Consumer*. T. Børresen, ed., Woodhead Publishing Ltd. (Accepted).

1. Introduction

Authorities in Denmark as well as in many other countries recommend that each person eat fish and seafood products at least twice a week or 200-300g on weekly basis. In Denmark and other developed regions, these recommendations are based primarily on the nutritional and health promoting effects of seafood (DVFA 2003; USDA 2005; EFSA 2005). However, several disadvantages such as dioxin and dioxin-like polychlorinated biphenyls (PCB's), accumulation of mercury (Wilson 2004; Anonymous 2004) and risk of diseases related to bacteria can be coupled to the consumption of fresh fish and seafood products (Huss *et al*. 2004). When authorities encourage to an increased consumption of fish, it is, at the same time, important to reduce health risks associated with these products. Microorganisms and viruses cause most diseases related to fish and seafood products. Listeriosis and botulism are among the most fatal diseases while, diseases caused by *Vibrio* spp., Salmonella, *Campylobacter* and viruses are less critical but much more frequent (Huss *et al.* 2004). Several microorganisms can form histamine, which is the causative agent in most outbreaks of reported seafood borne diseases in regions where statistics on this disease are performed (Todd 1997; Gillespie *et al*. 2001; Huss *et al.* 2004; CSPI 2006). However, in many countries histamine fish poisoning (HFP) is not a notifiable disease and they do not record and compile statistics on HFP. Consequently, HFP might be a more significant problem than actually seen in available statistics. The overall objective of this Ph.D. project has been to obtain information that can assist the process of reducing the frequency of HFP connected to ingestion of fresh fish and seafood products.

To reduce the formation of histamine in seafood an understanding of the mechanisms leading to its formation is central. In brief, endogenous histamine in small amounts is present in human blood and has important physiological effects. In addition, histamine is a bacterial metabolite formed by an enzymatic reaction from the amino acid histidine (Figure 1.1). When histamine is ingested in high concentrations (above 500-1,000 ppm) allergy-like symptoms and diseases occur. Histamine formation in concentrations high enough to cause disease is a problem in

certain fish species containing high concentrations of free histidine in the muscle tissue (Taylor 1986; Shalaby 1996; Lehane and Olley 2000; Huss *et al.* 2004).

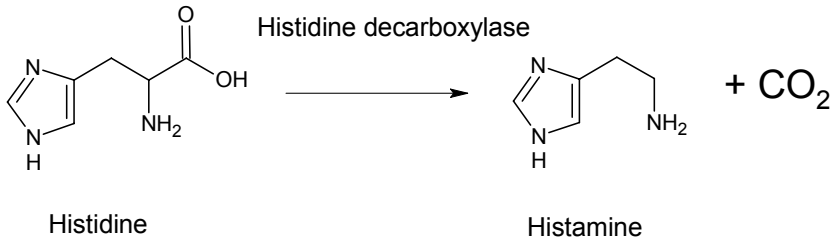


Figure 1.1 Decarboxylation of histidine into histamine by the enzyme L-histidine decarboxylase (EC 4.1.1.22).

It is well established that the enzyme (histidine decarboxylase, EC 4.1.1.22) involved in histamine formation, is produced by several species of mesophilic Enterobacteriaceae. Recently, also psychrotolerant bacteria have been associated with outbreaks of HFP (Kanki *et al*. 2004; Paper 1; Paper 2). Previously, numerous studies have been performed with mesophilic bacteria at elevated temperatures and it has been shown that at temperatures below 7-10°C these species do not produce histamine in toxic concentration since their growth is reduced significantly (Taylor 1986; Lehane and Olley 2000). Thus, prior to this Ph.D. project it was generally accepted that fish and seafood products involved in HFP had been exposed to elevated temperatures (see e.g. FDA, (2001c) and Kim *et al.*, (2004)). However, with an improved knowledge on the importance and technology of keeping the cool chain intact during transport and storage it is surprising that the numbers of HFP have not decreased (Paper 5). Consequently, it seems appropriate and necessary to change the attention from being exclusively on the mesophilic bacteria, to include the psychrotolerant histamine-producing bacteria (HPB) as well. Identification of a new psychrotolerant species of *Morganella* (Paper 3) has not only been crucial to this thesis but also added a new perspective to the understanding of HFP. *Phorobacterium phosphoreum* – another psychrotolerant HPB - is widely known for being the specific spoilage bacterium in fresh fish packed in modified atmosphere (Dalgaard 1995b). Recently, it has been recognised that histamine production by this bacterium can cause HFP (Kanki *et al.* 2004; Paper 1; Paper 2). However, this thesis mainly concerns *M. psychrotolerans*, therefore *P. phosphoreum* and the mesophilic group of histamine-producing bacteria are just briefly mentioned where appropriate even though their importance regarding outbreaks of HFP is recognised.

If the fish industry was able to predict histamine formation, they could state safe shelf-life for their fresh fish and seafood products and thereby minimise the risk of HFP and the economic losses that often accompanies outbreaks of HFP. A predictive model for histamine production that takes into account the effect of temperature, salt concentration and composition of the surrounding atmosphere could assist the industry in product optimisation. Predictive microbiology has been known for decades and this systematic accumulation and storage of knowledge can help manage food safety issues pro-actively (McMeekin and Ross 2002). Previously, so-called kinetic models that predict growth and metabolite (trimethylamine) formation in fresh fish has been suggested for H_2S producing *Shewanella* (Dalgaard 1995a; Dalgaard 2002). Gardini *et al.* (2001) developed a model for formation of the biogenic amines tyramine and 2-phenylethylamin by *Enterococcus faecalis* in milk. However, the use of predictive microbiology within the group of HPB has been limited. It is proclaimed, that it is not possible to predict histamine formation since it is unlikely to follow a growth curve and because metabolism of histamine by histaminase may be simultaneously (Lehane and Olley 2000). Torres *et al*. (2002) have in spite of this statement developed a model for histamine formation by the mesophilic *Morganella morganii,* in Chilean mackerel. The model is valid in a temperature range from 10-30°C. However the use of this model is rather restrictive since it is developed to predict a maximum concentration of 200 ppm histamine, whereas fish and seafood products causing HFP often contain more than 500-1,000 ppm (Paper 5). Acknowledging that the prediction of histamine formation in seafood is not an easy task the concept were tested and mathematical models for prediction of growth and histamine formation of *M. psychrotolerans* were developed (Paper 4). The effect of temperature, concentration of CO_2, a_w and pH is included in these models since they were the most obvious parameters influencing the formation of histamine.

In the present thesis, Chapter 2 concerns histamine fish poisoning, the symptoms and the products involved and summarises the current knowledge on histamine and other biogenic amines including the toxicology and detection. Chapter 3 reviews the present knowledge on HPB and the identification of *Morganella psychrotolerans.* In Chapter 4, the parameters affecting the formation of histamine are summarised leading to chapter 5, which describes the process of modelling the growth and histamine formation by *M. psychrotolerans*. The use of the developed

model is specified in chapter 6. Finally, the conclusions are presented in the last chapter, which also outlines the perspectives of the results obtained in the present thesis.

2. Histamine fish poisoning

Histamine poisoning is an intoxication caused by consumption of food with a high concentration of histamine. As this chapter will elucidate, seafood, especially finfish is the primary cause of histamine posioning. Only a few publications of histamine poisoning caused by foods other than seafood are available (Table 2.1). Thus, it seems as if histamine poisoning caused by food products other than seafood is insignificant. Consequently, the present thesis will be focused mainly on finfish and finfish products. As fish is the primary cause, the disease is often called histamine fish poisoning (HFP), a term which also will be used in the present thesis.

Table 2.1 Histamine poisoning related to products other than seafood.

Product	Histamine (ppm)	Country	Year	Cases	References
Gouda cheese	850	Netherlands	1967	1	Stratton *et al*. (1991); Self *et al*. (1999)
Sauerkraut	200	Germany	1971	1	Mayer and Pause (1972)
Swiss cheese	> 1,000	USA	1976	38	Stratton *et al*. (1991)
Swiss cheese	> 1,000	USA	1976	1	Stratton *et al*. (1991)
Swiss cheese	> 1,000	USA	1980	6	Taylor *et al.* (1982)
Gruyere cheese	300	France	1980-1983	4	Stratton *et al*. (1991)

Taylor (1986) did an excellent review on the toxicological and clinical aspects of HFP where previously publications on the topic were summarised. The work was followed by several reviews on the occurrence, formation, toxicology and detection of histamine (e.g. ten Brink *et al.* (1990); Stratton and Taylor (1991); Haláz *et al*. (1994); Bardocz (1995); Shalaby (1996) and Silla Santos (1996)). In 2000, Lehane and Olley published an extensive review of HFP in a risk assessment framework and recently Mavoratis and Quantick (2002a) added another review on HFP while Glória (2006) elaborated on bioactive amines, their physiological importance, metabolism, toxicological aspects and their occurrence in food. Many unanswered questions concerning the aetiology are repeated in these reviews, indicating that for decades, the area of HFP has been without significant progress.

Instead of HFP, some authors use the term scombroid fish poisoning that refers to fish from the families *Scombridae* (mackerel, tuna and bonito) and *Scomberesocidae* (saury) which contain high concentrations of histidine in the muscle tissue. However, non-scombroid fish (i.e. sardines and mahi-mahi) have been associated with this disease. Thus, the term scombroid fish poisoning seems inappropriate (Taylor 1986).

The issue of whether histamine is indeed the causative agent of HFP is still a matter of controversy, as several opposing arguments have been presented (see Chapter 2.4.1). However, it seems that histamine plays an important role, main or complementary, in the development of symptoms after ingestion of seafood containing more than 500-1,000 ppm histamine (Table 2.2).

Table 2.2 Overview of outbreaks (n=138) and cases (n=1749) of histamine fish poisoning as a function of the concentration of histamine (ppm) in different seafoods (Paper 5).

Histamine (ppm)	Outbreaks		Cases		Seafood
	Number	%	Number	%	
> 5,000	18	13	157	9	Escolar, kahawai, kingfish, marlin, saury, tuna, yellowfin tuna
1,000 – 5,000	62	45	897	51	Amberjack, anchovies, bluefish, cape yellowtail, castor oil fish/escolar, kahawai, mackerel, mahi-mahi, marlin, pilchard, red tuna, sailfish, sardines, swordfish, tuna
500 – 1,000	24	17	518	30	Anchovies, garfish, kahawai, mahi-mahi, mackerel, marlin, sardines, tuna
< 500	34	25	177	10	Anchovies, bonito, escolar, mackerel, mahi-mahi, pilchard, red tuna, sardines, skipjack, salmon, tuna

An outbreak of HFP is defined as two or more cases (one case = one diseased individual) linked by a common exposure, and showing typical symptoms such as; flushing, rash and headace (Table 2.3 and Table 2.4). The number of cases per outbreak of HFP is usually less than ten and often, single cases are observed (Figure 2.1, Table 2.3 and Table 2.4). Thus, in the present thesis, individual cases are included in the statistics with the same weight as outbreaks. The term incident is used, when both individual cases and outbreaks are included in figures and tables.

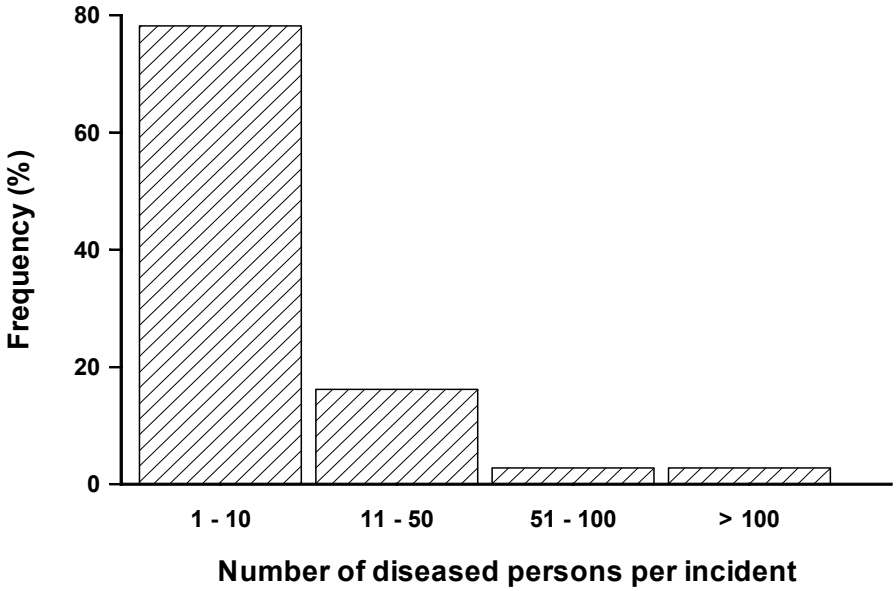


Figure 2.1 Frequency (%) of the number of diseased people observed per incident (n=139). Incidents include both outbreaks and individual cases. Data obtained from Emborg and Dalgaard (2007).

2.1 Symptoms of histamine fish poisoning

Recognition and correct diagnosis of HFP by the health care personnel is important to ensure that the correct treatment is given and to obtain reliable statistics about the occurrence of HFP. Knowledge and awareness of symptoms and their duration can help determine the diagnosis. HFP is considered a mild to moderate form of food poisoning with allergy-like symptoms. Case studies have shown that HFP symptoms begin within two minutes (meaning before the meal is finished) to within two hours after ingestion of the toxic food. For the majority of the incidents, the symptoms are relatively mild and resolve within a few hours (see references in Table 2.3 and Table 2.4). These observations were supported in the present study. The condition is rarely life-threatening and antihistamines are usually the only drug necessary for treatment and many cases are not treated at all (Taylor 1986).

Most HFP symptoms are easy to recognise. They can be cutaneous, gastrointestinal or neurological (Table 2.3 and Table 2.4). In addition, difficultly recognisable symptoms like hypotension occur but are typically not reported in case studies. It is difficult to compare observations of symptoms from different studies.

This is partly due to a lack of conformity in the vocabulary/questionnaires used when symptoms are recorded. In some studies, the symptoms observed within selected incidents are reported (Table 2.3). In others, the percentage of patients with specific symptoms in an outbreak is reported (Table 2.4). Symptoms vary little between outbreaks, eventhough different symptoms are observed between patients involved in the same outbreak (Arnold and Brown 1978). A relation between the symptoms of HFP and the concentration of histamine in the seafood is sometimes seen (Arnold and Brown 1978; Bartholomew *et al*. 1987; Feldman *et al*. 2005). The durations of the illness may be a function of the dose of exposure or the susceptibility of the individual patient (Taylor *et al*. 1989) but data on this are limited.

Table 2.3 Typical symptoms observed in relation to histamine fish poisoning and reported as percentage of incidents where the symptom in question was experienced.

	% of incidents			
	DK[a]	Finland[b]	UK[c]	Japan[d]
Number of incidents	15	17	94	14
Period	2004-2006	1975-1994	1976-1986	1951-1954
Cutaneous:				
Rash (bright red skin/urticaria)	73	18	48	36
Gastrointestinal:				
Nausea	33	53	29	21
Vomiting	27	35	32	50
Diarrhoea	53	29	48	36
Cramps (Stomach pain)	20	12	18	-
Neurological:				
Headache	20	35	39	71
Palpitation	27	18	3	14
Flushing	73	65	40	79
Oral blistering/burning/peppery taste/tingling	33	18	27	-
Itching	13	6	-	7
Others:				
Sweating	13	18	40	7
Swelling of the tongue	13	35	3	-
Dizziness	20	6	7	7
Tight chest	7	-	1	-
Respiratory distress	13	-	-	-
Facial swelling	13	-	3	-
Fever	13	6	-	-
Chills	-	-	-	36
Shaking and shivering	-	-	2	-
Throat swelling	-	12	-	-
Stiffness of muscles	-	6	-	-

[a] Emborg *et al*. (2006)
[b] Maijala *et al*. (1996)
[c] Bartholomew *et al*. (1987)
[d] Arnold and Brown (1978)

Table 2.4 Typical symptoms observed in relation to cases of histamine fish poisoning and reported as the percentage of patients experiencing the symptom in question. (Continued on next page.)

	% of cases					
	Australia [a]	Australia [b]	France [c]	South Africa [d]	Japan [e]	Switzerland [f]
Number of cases	7	9	20	22	12	31
Period	1990-1991	2001	1998	1990	1953	1972
Cutaneous:						
Rash (bright red skin/urticaria)	100	44	30	82	-	87
Gastrointestinal:						
Nausea	29	56	30	46	-	26
Vomiting	14	-	5	-	8	-
Diarrhoea	-	56	90	77	0	32
Cramps (Stomach pain)	-	44	30	-	0	-
Neurological:						
Headache	43	89	45	46	100	45
Palpitation	71	89	10	64	100	29
Flushing	71	87	-	-	100	-
Oral blistering/burning/peppery taste/tingling	14	-	-	27	-	-
Itching	-	-	-	32	-	-
Others:						
Sweating	71	-	-	-	-	-
Swelling of the tongue	-	-	-	-	-	-
Dizziness	14	-	10	-	92	-
Tight chest	-	-	-	-	-	3
Respiratory distress	-		-	23	-	-
Facial swelling	-	-	20	-	-	-
Fever	-	33	-	-	8	6
Chills	-	-	-	-	-	-
Shaking and shivering	-	-	-	-	-	-
Throat swelling	-	11	-	-	-	-
Stiffness of muscles	-	-	-	-	-	-

[a] Smart (1992)
[b] Leask *et al.* (2004)
[c] Boutin *et al.* (1998)
[d] Müller *et al*. (1992)
[e] Kawabata *et al*. (1955b)
[f] Marie *et al*. (1992)

Table 2.4 (Continued)

	% cases						
	USA [g]	USA [h]	USA [i]	Taiwan [j]	Taiwan [k]	Taiwan [k]	Taiwan [l]
Number of cases	95	15-17	42	115	4	48	94
Period	1973	1980	2003	1986	1996	1996	1997
Cutaneous:							
Rash (bright red skin/urticaria)	32	94	24	-	100	17	95
Gastrointestinal:							
Nausea	86	82	48	37	50	31	17
Vomiting	27	53	19	-	-	6	17
Diarrhoea	55	63	41	13	25	48	3
Cramps (Stomach pain)	71	41	31	-	-	29	17
Neurological:							
Headache	44	63	67	51	75	40	4
Palpitation	-	-	57	30	50	21	-
Flushing	46	65	62	62	-	-	-
Oral blistering/burning/peppery taste/tingling	63	100	24	-	-	4	-
Itching	-	-	12	-	-	-	-
Others:							
Sweating	-	-	33	-	100	46	-
Swelling of the tongue	-	24	14	-	-	13	-
Dizziness	-	24	48	78	100	58	4
Tight chest	-	-	-	-	-	19	-
Respiratory distress	-	-	19	-	-	-	-
Facial swelling	-	-	-	-	-	13	-
Fever	-	-	-	24	100	-	-
Chills	-	-	-	-	-	6	-
Shaking and shivering	-	-	-	-	-	-	-
Throat swelling	-	-	-	-	-	-	-
Stiffness of muscles	-	-	-	-	-	-	-

[g] Merson *et al*. (1974); 232 cases of which 95 were interviewed.
[h] Russell and Maretic (1986)
[i] Feldman *et al*. (2005)
[j] Kow-Tong and Malison (1987)
[k] Wu *et al*. (1997)
[l] Wu and Chen (2003)

The diagnosis of HFP should be based on the rapid onset of one or more typical symptoms and history of no previous allergies linked to the ingested food (Taylor 1986; Lehane and Olley 2000). It is, however, important that an international agreement on which symptoms are considered as typical is established. A typical progress of HFP can be as follows: At first, a flushing of the face and neck, accompanied by a feeling of heat and general discomfort. Often this is followed by an intense throbbing headache (Glória 2006). These symptoms were also among the most reported in relation to the present study (Table 2.3; Paper 5).

There seem to be no comprehensive studies evaluating if HFP symptoms differ between women and men. Data from Ijomah *et al.* (1991) and van Gelderen *et al*. (1992) suggest women may be more sensitive to HFP than men, but this has not been confirmed by outbreak statistics. Likewise, there is no foundation in the statistics to believe that younger and elderly people should be more sensitive than mid-age adults. Nevertheless, it is unusual that all individuals develop HFP symptoms even after comsumption of seafood with very high concentrations of histamine (Table 2.5; Table 2.6 and Table 2.7). Clinical studies are needed to determine if any susceptible populations' categories exist.

Table 2.5 Development of histamine fish poisoning after ingestion of seafood with high concentration of histamine. The data shown are reported incidents where the concentration of histamine, the number of patients and persons at risk were provided.

Country	Year	Number of people		Histamine in product (ppm)	Product	References
		Consuming product	Developing HFP symptoms (%)[a]			
France	1941	28	22 (79)	1,000-5,000	Tuna	Legroux *et al*. (1946)
Japan	1953	850	85 (10)	980	Seasoned mackerel	de las Rivas *et al.* (2006b)
Japan	1953	11	11 (100)	5,220	Dried saury	Kawabata *et al*. (1955b)
Japan	1954	400	90 (23)	970-1,070	Dried saury	Kawabata *et al*. (1955a)
Japan	1995	111	50 (45)	1,920-1,940	Tuna	Kawabata *et al*. (1955a)
USA	1968	9	8 (89)	4,255	Tuna	Schachner and Fodor (1968)
USA	1985	26	5 (19)	2,500	Bluefish	Etkind *et al*. (1987)
Japan	1998	40	21 (53)	400-7,300	Escolar	Kan *et al*. (2000)
USA	2003	56	42 (75)	2,000-3,800	Escolar	Feldman *et al*. (2005)
Denmark	2003	16	8 (50)	7,100-9,100	Tuna	Paper 1

[a] Attack rate calculated as: cases * 100 / persons at risk.

HFP is often mistaken for seafood allergy. It is, however, easy to distinguish between the two diseases. Symptoms experienced by several individuals (Table 2.5; Table 2.6 and Table 2.7) having no previous history of allergy combined with the rapid

onset after ingestion of fish or seafood products with high concentrations of histidine, are typical symptoms of HFP. Finally, the detection of histamine in elevated concentrations in the food remnants can confirm the diagnosis (Taylor *et al*. 1984; Taylor 1986; ten Brink *et al*. 1990; Stratton and Taylor 1991).

2.2 Occurrence of histamine fish poisoning

HFP is a worldwide health issue (Table 2.3 and Table 2.4) and in several countries, e.g. USA, Canada, England and Wales, outbreaks of HFP constitute the majority of seafoodborne diseases (Todd 1997; DeWaal *et al*. 2006; CSPI 2006; Hughes *et al*. 2007). The occurrence of HFP is most lilely underreported. This underreporting may be due to misdiagnosis (mistaken for seafood allergy), lack of mandatory reporting in several countries (Paper 5) and the mild nature of the disease.

2.2.1 Denmark

Related to the present Ph.D.-study, all reported incidents of HFP caused by seafood in Denmark during a three-year period, were analysed thoroughly. The product involved were characterised according to pH, salt and biogenic amines. Furthermore isolation of the dominating microflora present on remnants or so-called parallel samples were performed and the strongly histamine-producing bacteria (HPB) were identified (Table 2.6, Emborg *et al.* (2006)). This work was possible through collaboration between the Danish Institute for Fisheries Research and the Danish Veterinary and Food Administration (DVFA). Going through the official records of outbreaks of foodborne diseases from 1997 to 2003 (Table 2.7) and comparing these statistics with the information collected during the present study it seems the frequency of HFP in Denmark has been rather stable during the last ten years.

In Denmark, tuna, escolar and garfish primarily cause HFP (Table 2.6 and Table 2.7). Investigations of the official records also revealed that at least one outbreak of HFP reported to and registered by the Danish Veterinary and Food Administration in 2001 was not included in the official report (DVFA 2001b). This outbreak involved 20-25 children in a kindergarten and 4-5 adult personnel all served fried garfish for lunch. Analysis of the remnants showed a histamine concentration of 1,200 ppm (Kjølby 2005).

Table 2.6 Characteristics of the outbreaks of HFP in Denmark during 2004-2006. Data adapted from Emborg et al (2006)

Year	Products	Cases	Persons at risk	Attack rate (%)[a]	Biogenic amines (ppm)				Bacteria responsible for histamine formation
					Hist[b]	Cad[b]	Put[b]	Tyr[b]	
2004	Cold-smoked tuna	2	2	100	4,550	213	20	140	*Photobacterium phosphoreum*
2004	Cold-smoked tuna	1	12	8	1,973	132	6	88	*Morganella psychrotolerans*
2004	Tuna sandwich, canned tuna[c]	2	2	100	< 5	< 5	< 5	14	No strongly histamine-producing isolates
2004	Escolar[c]	7	-	-	< 5	< 5	< 5	< 5	No isolates
2004	Cooked escolar	4	< 50	> 8	4,090	257	< 5	17	No isolates
2004	Tuna heated in flexible film	8	-	-	6,432	286	40	< 5	*Morganella morganii* subsp. *morganii*
2004	Tuna (frozen and cooked)[c]	1	2	50	< 5	< 5	< 5	< 5	No isolates
2004	Cold-smoked tuna	10	< 65	> 15	914	68	< 5	23	No isolates
2004	Swordfish in saffron sauce	4	-	-	280-2,415	153	< 5	< 5	No isolates
2005	Escolar, marinated	7	15	47	5,810	321	< 5	< 5	No strongly histamine-producing isolates
2005	Tuna	2	-	-	96-1,738	< 5 - 112	< 5	< 5	No strongly histamine-producing isolates
2005	Smoked escolar	5	7	71	1,705	224	< 5	< 5	No strongly histamine-producing isolates
2005	Smoked tuna	6	< 68	>9	220	< 5	< 5	< 5	No isolates
2006	Canned tuna	1	1	100	335	55	13	39	No isolates
2006	Fresh tuna	2	2	100	1,050-1,750	80-110	7-8	54-56	No strongly histamine-producing isolates
2006	Fresh tuna	1	1	100	100-1,100	6-60	< 5	20-70	*Photobacterium phosphoreum*

[a] Calculated as: cases * 100 / persons at risk.
[b] Histamine (Hist), cadaverine (Cad), putrescine (Put) and tyramine (Tyr).
[c] For these incidences, no part of the product actually consumed was left for analysis and data was obtained by analysing a so-called parallel sample from the same batch/processor.

Table 2.7 Official records of outbreaks of histamine fish poisoning in Denmark (1997-2003)[a].

Year	Cases	Persons at risk	Attack rate (%)[b]	Product	Histamine (ppm)
1997	25	-	-	Garfish	High
	40	80	50	Garfish	Low
	3	-	-	Tuna	2,000
1998	2	2	100	Tuna	400-1,000
	6	12	50	Tuna	1,500
	3	3	100	Tuna	<50
	4	4	100	Tuna	1,000
1999	8	50	16	Tuna	2,000
	5	12	42	Escolar	1,200
2000	3	3	100	Swordfish	750-900
2001	3	4	75	Tuna	1,000
2002	No outbreaks were directly related to histamine. However, in three outbreaks with nausea, vomiting, abdominal cramps and diarrhoea, different tuna products were among the ingested food.				
2003	2	4	50	Tuna	3,000
	7	< 240	> 2	Blue marlin	-
	8	16	50	Tuna	3,000
	2	2	100	Tuna	High
	2	< 100	> 2	Escolar	-

[a] Data obtained from (http://www.foedevarestyrelsen.dk/fdir/publications/1998300/Rapport1.asp (in Danish) Accessed 11.07.2007; http://www.foedevarestyrelsen.dk/FDir/Publications/1999006/Rapport.htm (in Danish) Accessed 11.07.2007; DVFA 2000; DVFA 2001a; DVFA 2001b; DVFA 2004; DVFA 2005)
[b] Calculated as: cases * 100 / persons at risk.

2.2.2 Worldwide

In Paper 5, information about the occurrence of HFP worldwide was collected. It is important to stress that the data shown in Paper 5 does not represent a highly accurate picture of the incidents and cases of HFP. This is, as mentioned earlier, due to underreporting of HFP for several reasons. Comparison of the collected data from regions with different population size and for different recording periods was performed by the use of mean annual rate of HFP. Unfortunately HFP data for most of the heavily seafood consuming countries in the world are not avaiable. These countries include Tokelau (200 kg consumption of live weight of fish/year/person) and Niue (100 kg) in Oceania, Maldives (187 kg), Iceland (92 kg), Faeroe Island (87 kg), Saint Helena in Africa (85 kg) and Greenland (85 kg) (Laurenti 2004). Nevertheless, the collected data suggest the occurrence of HFP is highly variable. Denmark is among the contries with most incidents of HFP, even though the consumption of fish is moderat. There is, however, no clear relationship between the total amount of fish consumed and the frequency of HFP (Paper 5). There might be a relation between HFP and the type of fish

consumed. Fish species rich in free histidine (tuna, herring and mackerel) constitute ~52.5% of the fresh fish eaten in Denmark compared to 21.3% in Norway (herring and mackerel) (Welch *et al*. 2002). In Norway where cod and salmon is prefeered, occurrence of HFP is very low (Paper 5). The consumption of tuna and 13 other fishes being only ~9% of the total fish consumption. In Denmark, the consumption of tuna alone constitutes 6.8% (Welch *et al.* 2002).

2.3 Implicated seafood products

The large majority of histamine fish poisoning cases (>90%) are caused by seafood with a histamine concentration of more than 500 ppm (Table 2.2). It is interesting, however, that for 82% of the cases with a histamine concentration of less than 500 ppm, the histamine analyses were conducted on a fish from the same batch and not on the actual fish implicated in HFP. In England and Wales during 1987-1996 it was similarly observed that a very limited occurrence of HFP was due to seafood with low concentrations of histamine (Scoging 1998). Scoging (1998) found that 8% of the HFP incidents resulted from seafood with less than 200 ppm histamine, 13% from seafood with 200-1,000 ppm and 79% from seafood with more than 1,000 ppm histamine.

When no remnants from meals that cause outbreaks of HFP are available for analysis of histamine, fish from the same catch or products from the same batch are usually studied. Bartholomew *et al.* (1987) showed that in 47% of the fish from the same batch as those implicated in outbreaks, histamine concentrations were above 50 ppm histamine. They concluded that it is reasonable to analyse these fish when no other material is available. However, the concentration of histamine in spoiled fish can vary markedly between different specimen from a single batch as well as within a single fish (Table 2.8 and Paper 5). With this variability in mind, it is most important to distinguish between samples from seafood that actually caused HFP and other samples.

With respect to seafoods implicated in HFP it is interesting to note how this may change over time. In England and Wales for example, smoked mackerel caused 90% of 55 incidents during 1976-1979 whereas vacuum packed tuna steaks caused more than 50% of all the reported outbreaks in 1996 (Gilbert *et al*. 1980; Scoging 1998).

Table 2.8 Examples of variability in histamine concentrations between specimen from a single batch as well as within a single fish.

Fish species	Weight (kg)	Storage	Portions and histamine concentrations				References
			Portion 1	Histamine (ppm)	Portion 2	Histamine (ppm)	
Examples of seafood with considerable variability in histamine concentrations							
Dried saury	-[a]	-	Piece of fillet selected at random	2,700	Piece of fillet selected at random	200	Kawabata *et al*. (1955a)
Yellowfin tuna[b]	-	-	Fillet portion close to ventral cavity	4,380 ± 2,650	Fillet portion close to dorsal fin	303 ± 180	Lerke *et al*. (1978)
Skipjack	1.8-2.3	24 hours at 26.7°C	Fillet portion over ventral cavity	70-1,020	Fillet portion behind ventral cavity	16 - 45	Frank *et al*. (1981)
Smoked mackerel[b]	-	-	Fish-to-fish variation	960-1,750	Fish-to-fish variation	470 - 2,880	Ijomah *et al*. (1991)
Tuna	6	8°C	Not in tail	> 1,000	Tail	Below detection	Bédry *et al*. (2000)
Garfish	0.5	0°C	Piece of fillet selected at random	1,088	Piece of fillet selected at random	32	Dalgaard *et al*. (2006)
Garfish	0.5	5°C	Piece of fillet selected at random	2,439	Piece of fillet selected at random	358	Dalgaard *et al.* (2006)
Examples of seafood with little variability in histamine concentrations							
Spanish mackerel	-	275 hours at 0°C	Anterior	4	Posterior	5	Middlebrooks *et al*. (1988)
Spanish mackerel	-	90 hours at 15°C	Anterior	315	Posterior	310	Middlebrooks *et al.* (1988)
Spanish mackerel	-	32 hours at 30°C	Anterior	137	Posterior	67	Middlebrooks *et al.* (1988)
Tuna	50	15 days at 0°C	Anterior	450	Posterior	220	López-Sabater *et al*. (1988)
Tuna	50	5 days at 8°C	Anterior	2,000	Posterior	2,200	López-Sabater *et al.* (1988)
Tuna	50	36 hours at 20°C	Anterior	430	Posterior	920	López-Sabater *et al.* (1988)

[a] Not reported
[b] Sample from seafood implicated in an outbreak of histamine fish poisoning.

Tuna, escolar, kahawai and marlin are reported frequently to cause HFP. These fish naturally contain high concentrations of free histidine in their muscle tissue (Hibiki and Simidu 1959; Suyama and Yoshizaw 1973; Fletcher *et al*. 1995; Kan *et al*. 2000; Emborg *et al*. 2006; Paper 1). This free histidine can be decarboxylated by bacteria as discussed later (Chapter 3 and 4) and toxic concentrations can be formed. During 2004-2006 tuna was implicated in 69% of the Danish incidents, escolar in 25% and a single outbreak was caused by swordfish (Table 2.6).

Salmon contain a relatively low natural concentration (130-1090 ppm) of free histidine (Shirai *et al*. 1983; Espe *et al*. 1993; Emborg *et al*. 2002), however salmon has been implicated in incidents of HFP (Table 2.2). At least three incidents of HFP involving seven persons in all are reported to be caused by salmon (Bartholomew *et al.* 1987; Gessner *et al*. 1996). The concentration of histamine measured in these incidents were low (2-170ppm) and the persons involved must have been more susceptible to HFP than the average individual.

Besides typical concentrations of histamine above 500 ppm products involved in HFP contain a total concentration of other biogenic amines above 50 ppm (Table 2.6 and Table 2.9). Thus, a meal containing 100g seafood corresponds to an intake of above 50 mg histamine (with 100-500 being most common) and more than 5 mg of other biogenic amines (10-50 being most common).

2.4 Histamine and other biogenic amines

Biogenic amines are defined as: “Basic, non-volatile, nitrogenous compounds of low molecular weight which possess biological activity and are formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones” (Rice *et al*. 1976; Smith 1981; Askar and Treptow 1986; ten Brink *et al.* 1990; Silla Santos 1996; Rawles and Flick 1996).

Amines are formed and degraded during the normal metabolism of animals, plants and microorganisms and are therefore present in our food (Bardocz 1995). Dietary amines can be classified in several ways, i.e. based on chemical structure, biosynthetic pathway or physiological function (Bardocz 1995; Glória 2006). Based on their biosynthetic pathway, amines can be natural or biogenic. Natural, or endogenous, amines are formed during *de novo* biosynthesis from their precursors and stored in mast cells and basophiles while biogenic, or exogenous, amines are formed by bacterial

decarboxylation of free amino acids (Halász *et al*. 1994; Bardocz 1995; Shalaby 1996; Glória 2006). Some amines are both natural and biogenic (Figure 2.2). There is a general agreement among researchers that when it comes to seafood, the endogenous production of amines is insignificant when compared to the exogenous production (Arnold and Brown 1978; Taylor *et al.* 1984; Taylor 1986; ten Brink *et al.* 1990; Rawles and Flick 1996; Lehane and Olley 2000).

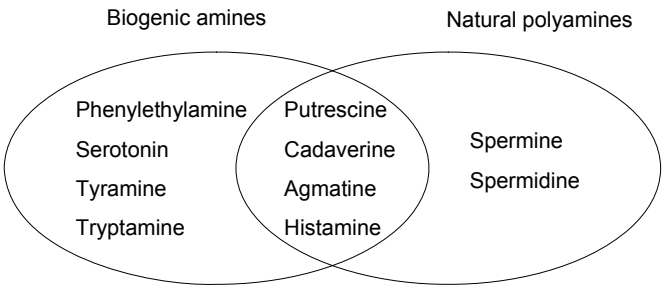


Figure 2.2 Amines commonly found in food. Some can be formed both as natural polyamines (during *de novo* biosynthesis) or by bacterial decarboxylation as biogenic amines.

The most important amines with regards to food safety and human health are histamine, putrecine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine and spermidine (Shalaby 1996). They are naturally present in a wide range of food products including fresh fish and seafood products, meat products, dairy products, wine beer, vegetables fruits, nuts and chocolate (Smith 1981; Askar and Treptow 1986; ten Brink *et al.* 1990; Halász *et al.* 1994; Bardocz 1995; Glória 2006). The present thesis focuses mainly on the occurrence of biogenic amines, specifically histamine in fresh fish and seafood products.

2.4.1 Toxicological response to histamine

For healthy people, ingestion of natural concentrations of biogenic amines present in food does not constitute any health risk (Rice *et al.* 1976; Glória 2006). However, growth to high concentrations of amine-producing bacteria (see Chapter 3) in food, either deliberately (as in fermented food such as fish sauce, sausages, cheese and sauerkraut) or due to accidental bacterial contamination, can have undesirable consequences as amines in high concentrations become toxic to humans (Smith 1981).

With respect to toxicity, histamine is by far the most important biogenic amine present in food. However, other biogenic amines can cause disease and discomfort (see reviews by Rice *et al.* (1976), Smith (1981), Taylor (1990) and Glória (2006)).

Histamine causes dilation of the peripheral blood vessels and capillaries, resulting in hypotension, flushing and headache. It also increases capillary permeability, which is related to oedema and urticaria. Furthermore, histamine-induced contraction of intestinal smooth muscle can explain the abdominal cramps, diarrhoea and vomiting sometimes observed in relation to HFP. The palpitations noted in relation to HFP may be due to increased heart rate and contractility caused by histamine (Taylor 1986; Lehane and Olley 2000; Parsons and Ganellin 2006). In brief, symptoms of HFP as previously reported correspond to known effects of histamine. The oral toxicity of histamine, however, is not entirely understood as discussed later in this section.

Histamine exerts its effects by interacting with receptors on cellular membranes. Four types of histamine receptors (H_1, H_2, H_3 and H_4) exist in different types of tissues (Parsons and Ganellin 2006). H_1- and H_2-receptors are important for HFP (Lehane and Olley 2000). Both intravenous and oral antihistaminic drugs have been developed to block these receptors and thereby the effects of the free histamine present in the blood. They include chlorpheniramine, diphenhydramine, hydroxyzine, promethazine (H_1-receptor antagonists) and cimetidine (H_2-receptor antagonist) (Kaliner *et al*. 1982; Blakesley 1983; Morrow *et al*. 1991; Ijomah *et al*. 1991; Smart 1992; Muller *et al*. 1992; Gessner *et al.* 1996; Parsons and Ganellin 2006).

In relation to outbreaks of HFP, it is unusual that all persons consuming the same finfish product develop symptoms of HFP. In fact, as shown in Table 2.6 and Table 2.7 a significant variation (10-80%) in the attack rate is observed. This means that even when seafood with very high concentration of histamine (e.g. 5,000 ppm) is eaten, not all consumers become ill (Table 2.5). Consequently, consumers' sensitivity to the agents responsible for HFP is highly variable. An unevenly distribution of histamine in the fish might also play a role (Table 2.8).

Table 2.9 Concentrations (ppm) of histamine and other biogenic amines in seafood involved in incidents of histamine fish poisoning.

Year	Seafood	Cases	Hist[a]	Put[a]	Cad[a]	Tryp[a]	Spm[a]	Spd[a]	Tyr[a]	Phe[a]	References
1973	Canned tuna	-	1,160	15	128	NT[b]	12	24	NT	NT	Kim and Bjeldanes (1979)
1984	Mahi-mahi	3	1,070-1,950	30-50	170-210	NT	20-50	30-40	50-150	NT	Yamanaka *et al*. (1987)
1985	Bluefish	5	2,500	300	740	NT	NT	NT	NT	NT	Etkind *et al*. (1987)
1991	Mackerel	-	1,178±673	< 5	110±79	NT	< 5	< 5	46±39	NT	Clifford *et al*. (1991)
1994	Sailfish	12	1,680-1,800	NT	110-145	1,850-2,080	200-500	125-175	NT	NT	Hwang *et al*. (1995)
1996	Salmon	1	0.3-2.4	6-8	0.6-4.2	NT	NT	NT	NT	NT	Gessner *et al* (1996)
1996	Marlin	3	841	<1	85	16	<1	<1	<1	<1	Hwang *et al*. (1997); Wu *et al* (1997)
1996	Tuna	48	1,185-2,719	NT	174-309	60-228	NT	NT	NT	NT	Wu *et al* (1997)
1998	Tuna	2	212	1	29	NT	NT	NT	NT	NT	Becker *et al*. (2001)
1998	Tuna	11	2,745-3,245	70	159	NT	NT	NT	NT	NT	Becker *et al*. (2001)
1999	Marlin	256	539-562	ND[c]-6	39-42	ND-3	ND-2	3-4	NT	ND	Su *et al*. (2000)
1999	Tuna	2	26-372	0.3-0.7	ND-21	NT	NT	NT	NT	NT	Becker *et al*. (2001)
2001	Canned mackerel	3	1,539	8	40	ND	90	ND	13	45	Tsai *et al*. (2005b)
2003	Tuna	8	7,100-9,100	14-16	27-54	NT	22-39	4-5	53-70	2-7	Paper 1
2004	Cold-smoked tuna	2	4,548±123	20±0.3	212±5	< 5	14±0	< 5	150±0	54	Paper 2
2004	Cold-smoked tuna	1	1,972±4	6±0.2	132±5	< 5	< 5	< 5	88±3	< 5	Paper 2
2004	Cold-smoked tuna	10	914±8	< 5	68±1	< 5	< 5	< 5	23±0.3	< 5	Paper 2
2004	Canned tuna[d]	2	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	14[d]	19[d]	Emborg *et al.* (2006)
2004	Escolar[d]	7	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	Emborg *et al.* (2006)
2004	Escolar	4	4,090	< 5	257	< 5	17	< 5	17	< 5	Emborg *et al.* (2006)
2004	Tuna	8	6,430	40	286	< 5	< 5	< 5	< 5	< 5	Emborg *et al.* (2006)
2004	Tuna[d]	1	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	< 5[d]	Emborg *et al.* (2006)
2004	Swordfish	4	208-2,415	< 5	153	< 5	< 5	< 5	< 5	11	Emborg *et al.* (2006)
2005	Escolar	7	5,810	< 5	321	< 5	< 5	< 5	< 5	< 5	Emborg *et al.* (2006)
2005	Tuna	2	1,738	< 5	112	< 5	< 5	< 5	< 5	< 5	Emborg *et al.* (2006)
2005	Smoked escolar	5	2,605-1,705	< 5	224-371	< 5	< 5	< 5	< 5	< 5	Emborg *et al.* (2006)
2005	Tuna	6	220	< 5	< 5	< 5	< 5	< 5	< 5	< 5	Emborg *et al.* (2006)
2006	Canned tuna	1	335-390	< 5-13	55-70	< 5	< 5-11	< 5	36-40	< 5-13	Emborg *et al.* (2006)
2006	Fresh tuna	2	1,050-1,750	7-8	79-110	< 5	14-15	< 5	54-56	< 5-8	Emborg *et al.* (2006)
2006	Fresh tuna	1	100-1,100	< 5	6-63	< 5	13-16	< 5	20-72	10-13	Emborg *et al.* (2006)

[a] Biogenic amines: Hist: Histamine, Put: Putrescine, Cad: Cadaverine, Tryp: Tryptamine, Spm: Spermine, Spd: Spermidine, Tyr: Tyramine, Phe: β-phenethylamine.
[b] Not tested.
[c] Not detected.
[d] For these incidences, no part of the product actually consumed was left for study and data was obtained by analysing a so called parallel sample from the same batch/processor.

It is not known if high or low resistance to histamine is constant for a given person or if this resistance may change over time as a function of the person's living habits. Clearly, when challenge studies to determine toxicity of histamine and other biogenic amines are carried out with a limited number of volunteers, it is important that these people are not all extreme in their sensitivity towards HFP. Apart from Clifford *et al.* (1991) and Ijomah *et al.* (1991), the challenge studies reported in the literature have not considered the sensitivity of the volunteers involved. This experimental limitation most likely contributes to the imprecise information available today concerning the toxicity of histamine and its importance for HFP (Paper 5).

Available data from challenge studies with volunteers suggest pure histamine and histamine added to seafood cannot always explain the toxicity of histamine-containing products. In one experiment 0.23 mg of histamine per kg body weight was reported to cause illness whereas as much as 3.3 mg histamine per kg body weight was consumed without symptoms by another person (Clifford *et al*. 1989). In a review, Taylor (1986) estimated that 1 mg histamine per kg body weight would cause human illness. The apparently low toxicity of histamine, as determined in some challenge studies, could simply be due to a combination of (i) sensitivity of the relatively few volunteers used and (ii) relatively low amounts of histamine (< 100-500 mg) evaluated (Motil and Scrimshaw 1979; Clifford *et al.* 1989; Clifford *et al*. 1991; Ijomah *et al.* 1991; Van Gelderen *et al*. 1992). However, different hypotheses have been proposed to explain the apparently low toxicity of histamine in challenge studies.

Instead of histamine as the causative agent of HFP, it has been suggested that other compounds could be responsible. Suggestion of toxins working as mast cell degranulators causing a release of histamine from the histamine-heparin complex in human mucosal mast cells in the gastrointestinal tract has been proposed (Arnold *et al*. 1980; Clifford *et al.* 1991; Ijomah *et al.* 1991). This hypothesis is consistent with the fact that antihistamine therapy eliminates symptoms of HFP. Lehane and Olley (2000) suggested cis-urocanic acid could be a mast cell degranulator responsible for HFP. Cis-urocanic acid is formed from L-histidine by the enzyme L-histidine ammonia lyase (HAL; EC 4.3.1.3; histidase), which is produced in several microorganisms (Shibatani *et al*. 1974; Hug and Hunter 1974; Mackie and Fernández-Salguero 1977; Baranowski 1985).

When mast cells are activated, histamine as well as tryptase and prostaglandin D_2 are released. It is therefore important that Morrow *et al*. (1991) and Sanchez-Guerrero *et al*. (1997) did not detect significant concentrations of these compounds or their degradation products in blood samples from patients with HFP. These data do not support the hypothesis that cis-urocanic acid or other mast cell degranulating agents should be of importance for HFP.

Is has also been suggested that compounds in seafood inhibit the normal metabolism/detoxification of histamine as further discussed in section 2.4.1.2.

2.4.1.1 Human metabolism of histamine

In humans, an oral dose of histamine is mainly (68-80%) excreted as histamine metabolites in the urine, whereas smaller portions can be recovered in faeces and exhaled as CO_2 from the lungs after degradation that may include the activity of intestinal bacteria (Sjaastad and Sjaastad 1974). Before histamine reaches the blood circulation, it is usually metabolised. In the small intestine (and maybe in the stomach), the enzyme histamine-N-methyltransferase (HMT, EC 2.1.1.8) transforms histamine and S-adenosylmethionine into N-methylhistamine, which can be further deaminated into methylimidazole acetaldehyde by a monoamine oxidase (MAO, EC 1.4.3.4) (Figure 2.3). Also, in the small intestine, the enzyme diamine oxidase (DAO, histaminase, EC 1.4.3.6) converts histamine into imidazole acetaldehyde (Figure 2.3). Histamine that crosses the intestinal wall is carried in the portal blood to the liver, where it is again metabolised by HMT and MAO (Hesterberg *et al*. 1984; Taylor 1986). Histamine intolerant individuals may have a deficiency of the enzyme DAO in the small intestine mucosa, resulting in decreased breakdown and increased absorption of histamine in the gastrointestinal tract (Rice *et al.* 1976).

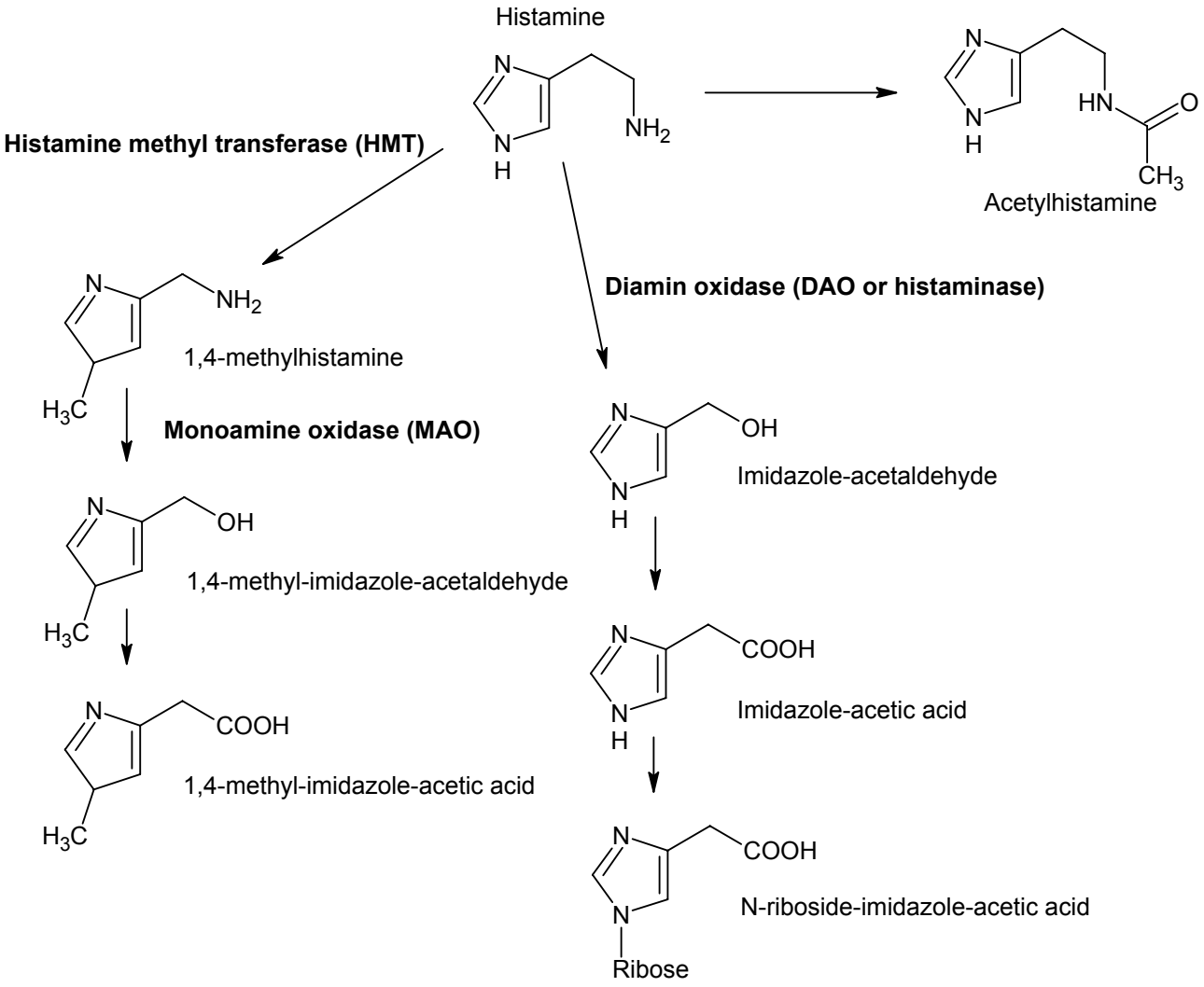


Figure 2.3 Normal metabolism or detoxification of histamine in humans. Modified from Glória (2006).

2.4.1.2 Inhibition of histamine metabolism

Numerous experiments with laboratory animals (rats, cats, dogs, guinea pigs, pigs and rabbits) have demonstrated that various compounds can reduce the normal histamine metabolism (Figure 2.3). Such compounds, or combinations of compounds, that reduce the activity of the enzymes DAO, HMT and MAO will increase the amount of histamine and methylhistamine in the blood circulation and thereby the oral toxicity of histamine (Taylor and Lieber 1979; Lyons *et al*. 1983; Hui and Taylor 1985; Satter and Lorenz 1990). Among the compounds with inhibiting abilities are agmatine, cadaverine, ß-phenylethylamine, putrescine, trimethylamine and tyramine, which are known to occur in spoiled seafood (Table 2.9). These findings suggest that the oral toxicity of histamine

in seafood may be potentiated by compounds that are present in spoiled but not in fresh seafood. This hypothesis could explain the apparently low toxicity of histamine in some challenge studies where the effect of histamine but no other compounds was evaluated (Motil and Scrimshaw 1979; Clifford *et al.* 1989; Ijomah *et al.* 1991). Cadaverine/histamine or putrescine/histamine ratios of 5:1 or greater in the duct of rats were required to inhibit histamine detoxification in vivo as assassed by measuring histamine metabolits in the urine (Hui and Taylor 1985). It has however not been possible to find such ratios in seafood that caused HFP (Table 2.9). Extrapolation of the quantitative effects from such data obtained with laboratory animals to humans is also difficult because the distribution of both the key enzymes of the normal histamine metabolism in tissues and the histamine receptors in tissues differ between humans and different species of laboratory animals (Buffoni 1966; Taylor 1986; Satter and Lorenz 1990; Lehane and Olley 2000).

Relatively few, controlled human volunteer studies have evaluated the effect of different compounds from food on the oral toxicity of histamine (Clifford *et al.* 1991; Van Gelderen *et al.* 1992). However, patients on drugs like isoniazid and tranylcypromine which are known to inhibit the enzyme DAO (Satter and Lorenz 1990) and possibly also MAO (Hauser and Baier 1982; Self *et al*. 1999), have increased sensitivity to seafoods with histamine or tyramine (Nuessle *et al*. 1965; Uragoda and Kottegoda 1977; Miki *et al*. 2005).

The volunteer studies of Clifford *et al*. (1991) and Van Gelderen *et al*. (1992) have clearly documented variation in the susceptibility towards histamine between human individuals. These studies are valuable, but more human volunteer studies are needed to evaluate the oral toxicity of histamine in combination with other compounds. It is particularly important to evaluate the effect of different realistic amounts of histamine (50-500 mg) in combination with different realistic amounts of other biogenic amines (5-50 mg) or other compounds from spoiled seafood (Table 2.6 and Table 2.9). In addition, it should be evaluated in challenge studies or in relation to HFP outbreaks, how seafood with low concentrations of histamine influences symptoms and concentrations of histamine, histamine metabolites, tryptase and prostaglandin D_2 in plasma and/or urine of patients/volunteers. This information would help determine if histamine potentiating compounds or endogenous histamine released from mast cells can explain HFP when caused by seafood with low concentrations of histamine. Although, histamine may not exclusively be responsible for HFP its presence in

elevated concentrations in seafood constitutes an excellent indicator that such products should not be consumed. (Lehane and Olley 2000).

2.4.2 Toxicity of biogenic amines other than histamine

Putrescine, spermine and spermidine play important roles in tumor growth (Glória 2006). Secondary amines such as putrescine and cadaverine can react with nitrite to form carcinogenic nitrosamines, nitrosopyrrolidine and nitrosopiperideine (Huis in´t Veld *et al*. 1990). However, next after histamine, monoamines like tyramine, phenylethylamine and tryptamine are the most problematic amines causing acute intoxication. They can cause a rise in blood pressure by releasing noradrenaline, constricting the vascular system and increasing the heart rate. This is known as the "cheese reaction", and these amines are called pressor amines. The potency of phenylethylamine is 10 times lower than tyramine but higher than tryptamine. Intoxication of these amines has an incubation time of from 10 minutes to 6 hours and symptoms include palpitations, severe headache, hypertension, flushing, profuse perspiration, stiff neck, nausea, vomiting and prostration. MAO-inhibitors, including several drugs, reduce the natural detoxification of the pressor amines and thus increase their oral toxicity (Rice *et al.* 1976; Smith 1981; Shalaby 1996; Glória 2006). The oral toxicity of tyramine is low and concentrations found in seafood (Table 2.9) will have no adverse effects on most consumers. However, for sensitive individuals, e.g. with reduced MAO activity due to medication or hereditary deficiency, very little tyramine can cause migraine headaches. For such individuals consumption of no more than 5 mg tyramine per meal has been recommended (McCabe 1986; Walker *et al*. 1996; Caston *et al*. 2002). Tyramine in high concentrations can be found in certain cheeses, wines, sausages, chicken liver and yeast extract. Chocolate is known to cause migraine for individuals susceptible to phenylethylamine (Maga 1978). In fish, the typical concentration of tyramine is less than 5 ppm, however in some incidents concentrations as high as 150 ppm has been observed. This corresponds to a typical intake of 0.5 mg histamine and in severe cases 15 mg.

2.5 Detection of biogenic amines

Standardised and harmonised analytical detection methods are needed to study and control the occurrence of biogenic amines in food. The earliest method for determination of histamine in seafood was a bioassay based on the fact that histamine causes contraction of guinea pig ileum (Geiger 1944). Later, various more accurate instrumental methods for detection of histamine and biogenic amines have been developed. In addition, sensory detection of histamine has been suggested. However, no single quantitative analytical procedure is able to quantify several biogenic amines simultaneously in different types of foods. This is mainly due to the presence of interfering compounds in the sample matrix and variability in the efficiency of extraction procedures for different types of samples (Moret and Conte 1996; Onal 2007).

Table 2.10 Sensory spoilage characteristics and concentrations of histamine in various seafoods.

Seafood	Sensory assessment	Temp. (°C)	Histamine (ppm)	References
Anchovy	Not spoiled[a]	Ambient	83 - 165	Kose and Erdem (2004)
Barracuda	Slight decomposition, but acceptable	32	15	Shakila *et al*. (2003)
Bluefish	Not spoiled[a]	5	116 ± 0	Gingerich *et al.* (1999)
Bluefish	Not spoiled[a]	10	43 ± 34	Gingerich *et al.* (1999)
Bluefish	Not spoiled[a]	15	286 ± 261	Gingerich *et al.* (1999)
Garfish	Not spoiled[a]	0	0 - 13	Dalgaard *et al.* (2006)
Garfish	Not spoiled[a]	5	28 - 509	Dalgaard *et al.* (2006)
Mackerel	Slightly putrefied	5	280	Okuzumi *et al.* (1984c)
Mackerel	Slightly putrefied	10	32	Okuzumi *et al.* (1984c)
Mackerel	Slightly putrefied	15	261 - 453	Okuzumi *et al.* (1984c)
Mackerel	Slight decomposition, but acceptable	32	90	Shakila *et al*. (2003)
Mahi-mahi	Initial decomposing	13	0 - 1	Du *et al.* (2001)
Mahi-mahi	Not spoiled	15 -25	800 – 2,500	Fletcher *et al.* (1995)
Trevally	Slight decomposition, but acceptable	32	52	Shakila *et al*. (2003)
Tuna	Not spoiled[a]	0	20 ± 12	Veciana-Nogúes *et al.* (1997)
Tuna	Not spoiled[a]	0	220 – 1,200	López-Sabater *et al.* (1996b)
Tuna	Not spoiled[a]	8	2,500	López-Sabater *et al.* (1996b)
Tuna	Not spoiled[a]	8	110 ± 30	Veciana-Nogúes *et al.* (1997)
Tuna	Not spoiled[a]	20	924 ± 21	Veciana-Nogúes *et al.* (1997)
Sailfish	Not spoiled		1,680 – 1,800	Hwang *et al.* (1995)
Sardine	Slight decomposition, but acceptable	32	45	Shakila *et al*. (2003)
Sardine	Moderately fresh	0	80 - 90	El-Marrakchi *et al.* (1990)

[a] Samples near the limit of sensory acceptance judged by a sensory panel

2.5.1 Sensory detection

Histamine in toxic concentrations can be formed in seafood before products appear sensory spoiled (Table 2.10). The authority in Denmark, however, uses a trained sensory panel on routine basis as a preliminary detection of histamine in canned tuna. When positive in the sensory analysis products are then further analysed by high performance liquid chromatography (HPLC) for confirmation and quantification (EC 2005; DVFA 2007). Numerous HFP incidents have been due to seafood with more than 1,000 ppm histamine (Table 2.2). This clearly demonstrates the inability of many consumers to detect even high concentrations in histamine seafoods. It seems sensory detection of histamine can be successful when trained and experienced paneliest evaluate a specific product wherease in other cased sensory detection of histamine is not reliable.

2.5.2 Instrumental detection

All analytical methods for determination of biogenic amines in proteinaceous foods involve two main steps; (i) extraction of the biogenic amines from the matrix and (ii) determination of the biogenic amines. For histamine analysis in food, HPLC is the most often-used analysis method. (Onal 2007). In addition, several enzymatic methods have been developed for histamine analysis. These rely e.g. on the enzyme histamine-N-methyltransferase and radioactive S-adenosylmethionine and they can be much more sensitive than required with concern to toxic concentrations of histamine in seafood (Taylor 1986; Stratton and Taylor 1991; Lehane and Olley 2000). Enzymatic kits with good reproducibility, which are designed for seafood, are available (Rogers and Staruszkiewicz 2000). However, the cost of these kits for routine testing of numerous samples for quality assurance or preventive-testing can be high (Lehane and Olley 2000).

EU-regulations (EC 2073/2005) indicate a reference HPLC method for analysis of histamine in seafood (EC 2005). This method detects histamine, putrescine, cadaverine, spermine and spermidine and the procedure is indicated to be suitable for all fish species. The amines are extracted with perchloric acid, reacted with dansyl chloride and then separated by HPLC (Malle *et al*. 1996).

The Association of Official Analytical Chemists procedure (AOAC 2005) is the official method of analysis of histamine in foods in the U.S. Histamine is extracted from

seafood with methanol, which is passed through an ion exchange column and eluted with HCl. The elute is collected and mixed with a solution of o-phthalaldehyde (OPA) and the fluorescence intensity is measured

The concentration of biogenic amines in fresh seafood is very low. At the same time microbial spoilage of seafood frequently results in biogenic amines formation. Thus, concentrations of biogenic amines may serve as a useful indicator of spoilage (Halász *et al.* 1994). In fact, the concentration of histamine and other biogenic amines have been tested as single or multiple compound quality indices (Mietz and Karmas 1977; Dawood *et al*. 1988; Yamanaka 1989; Yamanaka *et al*. 1989; Sims *et al*. 1992; Rawles and Flick 1996; Veciana-Nogués *et al*. 1997; Jørgensen *et al*. 2000a). Costs of biogenic amine analyses and accuracy of the suggested indices, however, limit their usefulness for routine screenings (Lehane and Olley 2000). Simple, rapid, inexpensive and accurate methods for quantification of histamine and other biogenic amines are needed in this area.

3. Histamine-producing bacteria

Information about histamine-producing bacteria (HPB) in seafood is important. This information can be used e.g. to reduce their growth and histamine formation as well as for specific detection of the the relvant bacteria.

3.1 Histidine decarboxylase

Histamine in seafood is primarily formed by bacteria that produce an active L-histidine decarboxylase (HDC). In living bacteria, HDC functions in cooperation with a membrane exchanger that allows histidine to be transported into the cell and histamine to be transported out of the cell (Molenaar *et al*. 1993). The function of histamine in bacterial metabolism is, however, unknown. It has been suggested that production and excretion of histamine may generate metabolic energy or be involved in the acid stress response (Van Poelje and Snell 1990; Molenaar *et al.* 1993; Lucas *et al*. 2005). Decarboxylation of histidine releases CO_2 and consumes a proton; hence, decarboxylation can supply nutritionally essential CO_2 and neutralise acids and thus, participate in the regulation of the intracellular pH (Van Poelje and Snell 1990; Molenaar *et al.* 1993). The formation of histamine may possibly alter the physiology of the host to the bacteria's advantage. For example, since histamine is a vasodilator, its formation could result in transfer of essential nutrients from the host to the bacteria (Van Poelje and Snell 1990).

Histidine is the only amino acid so far known for which decarboxylases of two different types have evolved (Tanase *et al*. 1985; Van Poelje and Snell 1990). One type is the pyridoxal 5'-phosphate-dependent HDC. This HDC has been isolated and characterised from the following Gram-negative bacteria: *Morganella morganii* (Tanase *et al.* 1985), *Raoultella planticola* (Guirard and Snell 1987; Kanki *et al*. 2007), *Enterobacter aerogenes* (Guirard and Snell 1987), *Photobacterium phosphoreum* (Morii and Kasama 1995; Morii and Kasama 2004; Kanki *et al.* 2007) and *Photobacterium damsela* (Kanki *et al.* 2007).

The other type of HDC is the pyruvoyl-dependent HDC produced by Gram-positive bacteria. This enzyme is for example isolated from the following bacteria: *Lactobacillus* 30a (Hackert *et al*. 1981), *Lactobacillus hilgardii* 0006 (Lucas *et al.* 2005), *Leuconostoc oeni* IOEB (Coton *et al*. 1998), *Tetragenococcus muriaticus* (Konagaya *et al*. 2002) and *Clostridium perfringens* (Huynh and Snell 1985). Both types of HDC are inducible but show no sequence homology or relatedness in amino acid structure (Hackert *et al.* 1981; Tanase *et al.* 1985; Vaaler *et al*. 1986; Van Poelje and Snell 1990; Kamath *et al*. 1991). No organism able to produce HDC of both types is known yet (Van Poelje and Snell 1990). The Enzyme Commission makes no distinction between the pyruvoyl-dependent and the pyridoxal 5'-phosphate-dependent L-Histidine decarboxylase (HDC, EC 4.1.1.22) (Vaaler *et al.* 1986).

The presence of HDC in one strain does not necessarily mean that other strains of the same species also produce HDC or that the activities of the HDCs are equal (Taylor *et al*. 1978; Yoshinaga and Frank 1982; Kim *et al*. 2001c; Wauters *et al*. 2004). One proposed explanation of the variability in histamine production is that HDC can be controlled by a plasmid that may be transferred from strain to strain, from species to species or from genus to genus (Tolmasky *et al*. 1995; Lehane and Olley 2000). A plasmid-borne HDC located at least on two different plasmids has been identified in *Vibrio anguillarum* (Tolmasky *et al.* 1995; Barancin *et al*. 1998). Indirectly, the production of histamine influences the virulence of this fish pathogen. HDC is necessary for the production of a siderophore anguibactin, which is required for iron uptake by *V. anguillarum.* Without the uptake of iron, *V. anguillarum* becomes avirulent (Tolmasky *et al.* 1995). However, HDC seems to play a completely different role in *V. anguillarum* as compared to other organisms. It needs to be noted that *V. anguillarum* never has been identified as the responsible HPB in relation to outbreaks of HFP (Table 3.1).

Another explanation for variability in histamine formation between strains of bacteria might be the presence of two different HDCs: An inductive HDC and a constitutive HDC. Both inductive and constitutive decarboxylases have been identified for ornithine and arginine (Tabor and Tabor 1985). The constitutive HDC, however, is known for *P. phosphoreum* only and the corresponding gene is not identified (Morii and Kasama 1995).

3.2 Gram-positive histamine-producing bacteria

Strains of some Gram-positive bacteria can produce histamine and they have been isolated from salted, dried or fermented foods (Landete *et al*. 2006). *Staphylococcus* spp., *T. muriaticus, Cl. perfringens* and some strains of *Lactobacillus* spp. are among the best known Gram-positive histamine producers (Taylor *et al.* 1978; Yoshinaga and Frank 1982; Hernández-Herrero *et al*. 1999; Kobayashi *et al*. 2000; Lucas *et al.* 2005). Gram-positive HPB have never been isolated from fish or seafood products that caused HFP (Table 3.1). Therefore Gram-positive histamine producing bacteria will not be discussed separately in the present thesis but some aspects of histamine formation will be compared between Gram-positive and Gram-negative bacteria.

3.3 Gram-negative histamine-producing bacteria

A wide range of Gram-negative bacteria isolated from seafood is able to produce histamine. However, only a minor part of them is able to produce histamine in high concentrations (> 1,000 ppm) even at optimal conditions (Table 3.2). These bacteria have been designated prolific histamine producers (Behling and Taylor 1982).

Until 2004, a very limited number of studies had identified the bacteria responsible for histamine formation in seafood that had caused HFP (Table 3.1). These few studies showed that HFP was caused by the activity of mesophilic histamine-producing bacteria. However, knowledge about psychrotolerant HPB has to some degree been available since the 1960's when Kimata (1961) observed histamine production in toxic concentrations at 6-7°C.

Later, histamine production in naturally contaminated seafood stored between 0-4°C was observed in many studies (Okuzumi *et al*. 1982; Van Spreekens 1987; Morii *et al*. 1988; Ababouch *et al*. 1991a; Ababouch *et al*. 1996; López-Sabater *et al*. 1996b; Silva *et al*. 1998; Kanki *et al.* 2004; Paper 1; Paper 2).

Table 3.1 Incidents of histamine fish poisoning for which the microorganisms responsible for histamine formation in the products have been identified and reported. (Modified from Paper 5).

Year	Country	Products	Cases	Histamine (ppm)	Bacteria responsible for histamine formation	References
1955	Japan	Fresh tuna	50	1,200	*Morganella morganii*	Kawabata *et al*. (1955b)
1965	Japan	Fresh tuna	NR[a]	NR	*Morganella morganii*	Sakabe (1973)
1967	Czechoslovakia	Fresh tuna	NR	120-3,100	*Hafnia spp.*	Havelka (1967)
1977	USA	Fresh tuna	15	1,600-9,190	*Raoultella planticola*	Lerke (1978)
2002	Japan	Dried sardines	1	3,000	*Photobacterium phosphoreum*	Kanki *et al*.(2004)
2003	Denmark	Tuna in chilli sauce	8	7,100-9,100	*Morganella psychrotolerans, Photobacterium phosphoreum*	Paper 1
2004	Denmark	Cold-smoked tuna	2	4,500	*Photobacterium phosphoreum*	Paper 2
2004	Denmark	Cold-smoked tuna	1	2,000	*Morganella psychrotolerans*	Paper 2
2004	Denmark	Tuna heated in flexible film	8	6,400	*Morganella morganii*	Emborg *et al*. (2006)
2006	Denmark	Fresh tuna	1	1,100	*Photobacterium phosphoreum*	Emborg *et al*. (2006)

[a] Not reported (NR).

Today, it is clear that both mesophilic bacteria including *M. morganii*, *Hafnia* and *Raoultella planticola* and psychrotolerant bacteria including *M. psychrotolerans* and *P. phosphoreum* are important for histamine formation in seafood HFP (Table 3.1; Paper 1; Paper 2; Paper 3; Paper 4 and Paper 5). During the last five years, more psychrotolerant than mesophilic bacteria have been isolated from seafood that caused HFP. This can be a result of improved chilled storage conditions or increased attention to psychrotolerant organisms (Paper 5).

Members of the Enterobacteriaceae are often identified as histamine producers (Table 3.2). However, in a study by Wauters *et al.* (2004) only 5 species of 37 tested were positive (*M. morganii, R. planticola, R. ornithinolytica, Citrobacter youngae* and *E. aerogenes*). This might be explained by (i) natural variation among the species, (ii) less sensitive test used by Wauters *et al.* (1987) compared to other studies or (iii) misidentification of the bacteria in the earlier studies.

3.3.1 *Morganella psychrotolerans*

Until 2006, *M. morganii* was the only species known within the genus *Morganella* (See describtion in Chapter 3.3.2). However, during the 1950's, a *Morganella*-like bacterium initially named *Achromobacter histamineum* was studied in Japan (Kimata 1961). In contrast to *M. morganii, A. histamineum* had a growth-optimum between 20-25°C and did not grow at 37°C. Similar to *M. morganii*, *A. histamineum* were capable of histamine formation in high concentrations. Nevertheless, *A. histamineum* was renamed *M. morganii* due to many common biochemical properties (Kimata 1961).

In 2006, a new species of *Morganella* was identified and named *Morganella psychrotolerans* (Paper 3). This new species is like *A. histamineum* unable to grow at 37°C and importently *M. psychrotolerans* can grow and produce toxic concentration of histamine at 0°C (Fig. 3.1). It has not been reported if *A. histamineum* has these properties. The isolation and identification *M. psychrotolerans* is most important as the existence of a psychrotolerant and strongly histamine-producing bacteria can explain many incidents of HFP. The identification of psychrotolerant HPB capable of histamine formation at 0°C will propably reduce the commonness use of temperature abuse as the explanation for histamine formation.

Table 3.2 Examples of prolific Gram-negative mesophilic histamine-producing bacteria and their origin.

Organism	Source	References
Acinetobacter baumannii	Sailfish (*Istiophorus platypterus*)	Tsai *et al.* (2004)
Citrobacter freundii	Tuna (*Thunnus thynnus*)	López-Sabater *et al*. (1996b)
Citrobacter braakii	Albacore	Kim *et al.* (2001b)
Enterobacter aerogenes	Albacore, Indian anchovy (*Stolephorus indicus*), Sailfish (*Istiophorus platypterus*), tuna	Kim *et al.* (2001b); Takahashi *et al.* (2003); Tsai *et al.* (2004) and Rodtong *et al*. (2005)
Enterobacter agglomerans	Tuna (*Thunnus thynnus*)	López-Sabater *et al.* (1996b)
Enterobacter cloacae	Albacore tuna (*Thunnus alalunga*), tuna *Thunnus thynnus)*	López-Sabater *et al.* (1996b) and (Kim *et al.* 2001c)
Hafnia	Tuna	Ferencik (1970)
Hafnia alvei	Tuna (*Thunnus thynnus),* skipjack tuna	Arnold *et al*. (1980) and López-Sabater *et al.* (1996b)
Klebsiella oxytoca	Albacore, tuna (*Thunnus thynnus),* sailfish (*Istiophorus platypterus*)	López-Sabater *et al.* (1994b); López-Sabater *et al.* (1996a; 1996b); Kim *et al*. (2001b) and Tsai *et al*. (2004),
Klebsiella pneumoniae	Tuna (*Thunnus thynnus*), jack mackerel (*Trachurus symmetricus*)	Taylor and Speckhard (1984) and López-Sabater *et al.* (1996b)
Morganella morganii	Albacore tuna (*Thunnus alalunga*), anchovies (*Engralis encrasicholus*), bluefish (*Pomatumus saltatrix*), big eye tuna (*Thunnus obesus*), bluefin tuna (*Thunnus thynnus*), horse mackerel, Indian anchovy (*Stolephorus indicus*), jack mackerel (*Trachurus symmetricus*), mackerel (*Scomber scombrus*), mahi-mahi (*Coryphaena hippurus*), Pacific mackerel (*Scomber japonicus*), sardine (*Sardina pilchardus*), skipjack tuna (*Katsuwonus pelamis*), yellowtail, yellow fin tuna (*Thunnus albacares*)	Aiiso *et al.* (1958); Arnold *et al*. (1980); Leitão *et al.* (1983); Taylor and Speckhard (1983); Taylor and Speckhard (1984); Frank *et al*. (1985), Ababouch *et al*. (1991b); Oka *et al.* (1993); Rodriquez-Jerez *et al.* (1993); López-Sabater *et al.* (1994a; 1994b); Rodriquez-Jerez *et al.* (1994); Lopez-Galvez *et al*. (1995); López-Sabater *et al.* (1996a; 1996b); Gingerich *et al*.(1999); Kim *et al*. (2000); Gingerich *et al.* (2001); Kim *et al.* (2001a; 2001b; 2001c); Lorca *et al*. (2001), Kim *et al*. (2002); Takahashi *et al.* (2003); Rodtong *et al*. (2005); and Paper 3
Photobacterium damsela	Horse mackerel	Takahashi *et al.* (2003)
Photobacterium legionathi	Indian mackerel (*Rastrelliger kanagurta*), Indian oil sardine (*Sardinella longiceps*)	Ramesh *et al.* (1989)
Proteus mirabilis	Mahi-mahi (*Coryphaena hippurus*) sardine (Sardina pilchardus)	Frank *et al*. (1985), Ababouch *et al*. (1991b)
Proteus penneri	Sailfish (*Istiophorus platypterus*)	Tsai *et al.* (2004)
Proteus vulgaris	Albacore tuna, Indian anchovy (*Stolephorus indicus*), Pacific mackerel (*Scomber japonicus*), sailfish (*Istiophorus platypterus*), skipjack tuna (*Katsuwonus pelamis*), horse mackerel, young yellowtail	Arnold *et al*. (1980); Kim *et al* (2001a); Kim *et al*. (2001c); Takahashi *et al.* (2003); Tsai *et al.* (2004); and Rodtong *et al*. (2005)
Proteus vulgarius[a]	Sailfish (*Istiophorus platypterus*)	Tsai *et al.* (2004)
Rahnella agnatilis[a]	Sailfish (*Istiophorus platypterus*)	Tsai *et al.* (2004)
Raoultella ornithinolytica	Tuna, bonito and sardines	Kanki *et al.* (2002)
Raoultella planticola	Tuna, bonito and sardines	Kanki *et al.* (2002)
Raoultella planticola	Horse mackerel, tuna, bigeye tuna, autum albacore	Takahashi *et al.* (2003)
Serratia fonticola	Albacore, bluefin tuna (*Thunnus thynnus*)	López-Sabater *et al.* (1996b) and Kim *et al.* (2001b)
Serratia marcescens	Tuna (*Thunnus thynnus*)	López-Sabater *et al.* (1996b)
Vibrio fischeri	Indian mackerel (*Rastrelliger kanagurta*), Indian oil sardine (*Sardinella longiceps*)	Ramesh *et al.* (1989)
Vibrio harveyi	Indian mackerel (*Rastrelliger kanagurta*), Indian oil sardine (*Sardinella longiceps*)	Ramesh *et al.* (1989)

[a] Tsai *et al*., 2004 reported these bacteria, however, they are assumed to be misspelling versions of: *P. vulgaris* and *R. aquatillis.*

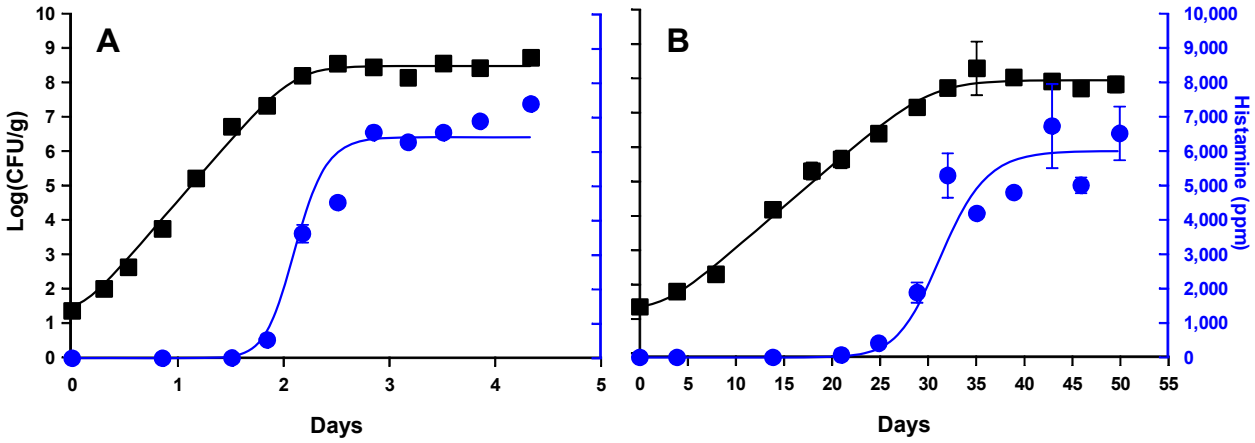


Figure 3.1 Growth (■) and histamine production (●) by *Morganella psychrotolerans* in amino acid-enriched LB Miller broth stored at A) 20°C and B) 0°C. Adapted from Paper 4.

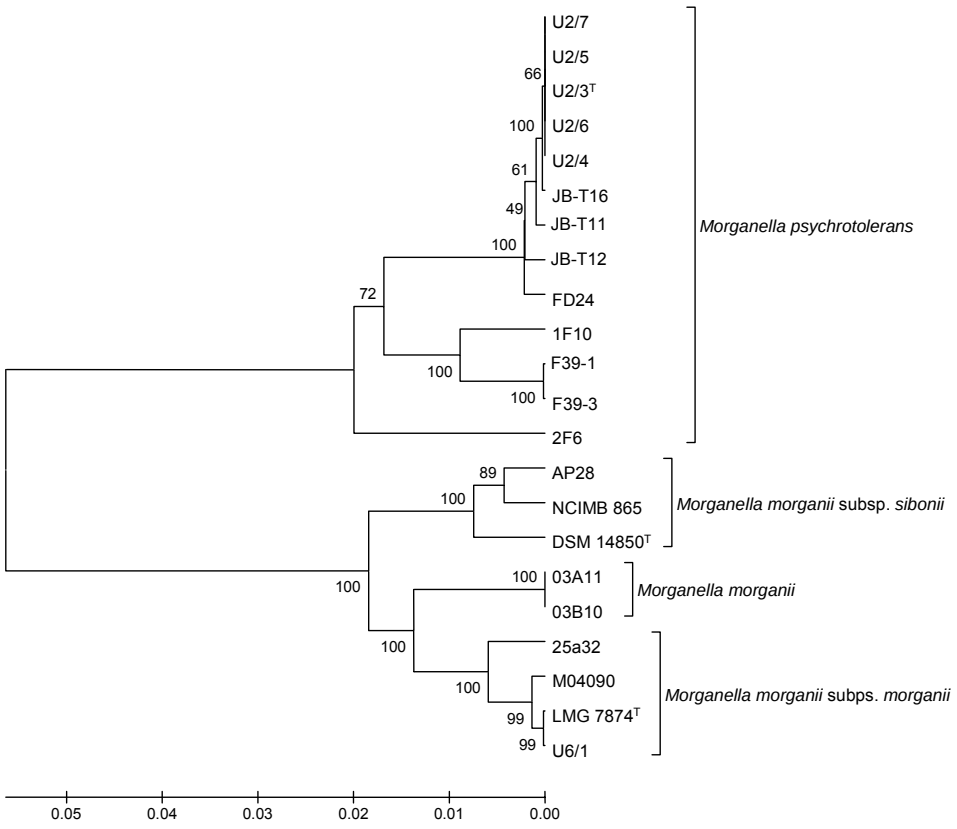


Figure 3.2 Neighbour joining tree (Kimura two-parameter) showing the relationship between merged sequences (3557 bp) of seven housekeeping gene fragments from 22 *Morganella* strains. Bootstrap values from 1000 replicates appear next to the corresponding branch. Bar, nucleotide substitutions per site (Paper 3).

Besides being isolated from two outbreaks of HFP, *M. psychrotolerans* has been found on garfish and tuna (Dalgaard *et al*. 2006; Paper 1; Paper 2). The habitat and occurrence of this psychrotolerant and histamine-producing species is, however, still unknown and deserves more attention.

At both 0°C and 20°C *M. psychrotolerans* produced histamine in significant concentrations when the concentration of cells reached about 10^6 CFU/g (Figure 3.1). As for *M. morganii, M. psychrotolerans* produces no biogenic amines other than histamine in high concentrations when grown in amino-enriched broth with an amino acid profile resembling the profile in tuna (Ryser *et al*. 1984; Kim *et al*. 2000; Paper 1). All the isolated strains of *M. psychrotolerans* form histamine in high concentrations. A polyphasic characterisation with both multi locus sequencing and traditional phenotypical/biochemical tests were used to differentiate between *M. morganii* and *M. psychrotolerans* (Paper 3). Twenty-two strains collected within a broad time range (1920-2004), from different geographical locations and habitats were included in the study. Fragments of seven housekeeping genes were selected for sequencing.

88.7% sequence similarity was found between the group means of the merged housekeeping genes from *M. psychrotolerans* and *M. morganii* (Figure 3.2*).* This clustering was supported by DNA-DNA hybridisation and a limited number of phenotypical/biochemical tests. In contrast to *M. morganii*, *M. psychrotolerans* is capable of growth at 2°C (and even 0°C as shown previously in Figure 3.1). *M. morganii* is more tolerant towards high concentrations of NaCl, as it grows with 8.5% at 25°C. *M. psychrotolerans* do not grow with more than 6% NaCl when tested at 10°C (Paper 4). Finally, D-galactose is fermented by *M. morganii* but not by *M. psychrotolerans* (Table 3.3; Paper 3). It seems possible that *M. psychrotolerans,* like *M. morganii,* can be divided into subspecies. This is supported by sequence data and phenotypic data (Figure 3.2; Table 3.3) but more isolates should be studied to validate the observed subgroups within *M. psychrotolerans*.

Table 3.3 Phenotypic characteristics of *Morganella psychrotolerans*, *M. morganii* subsp. *morganii* and *M. morganii* subsp. *sibonii* (Modified from Paper 3).

	Percentage of positive results				
	M. psychrotolerans	*M. psychrotolerans*	*M. morganii* subsp. *morganii*	*M. morganii* subsp. *sibonii*	*M. morganii*
Number of isolates	9	4	4	3	2
Origin	Tuna	Lumpfish roe and garfish fillets	Seafood and clinical isolates	Seafood and clinical isolates	Smoked fish
Lysin decarboxylase[a]	0	0	0	33	0
Ornithin decarboxylase[a]	100	50	100	100	100
Urea[a]	100	100	75	100	100
L-tryptophan-TDA[a]	88	75	100	100	100
Citrate utilisation[a]	0	25	0	0	0
Acid from D-trehalose[b]	0	50	0	100	100
Acid from D-galactose[b]	0	0	100	100	100
0°C	100	50	0	0	0
2°C	100	100	0	0	0
4°C	100	100	100	100	100
35°C	100	100	100	100	100
37°C	0	0	100	100	100
0% NaCl[c]	100	100	100	100	100
7.5% NaCl[c]	22	50	100	100	100
8.5 % NaCl[c]	0	0	100	100	100
10% NaCl[c]	0	0	25	30	50
pH 4.1[c]	0	0	0	0	0
pH 4.6[c]	88	75	100	100	100
pH 9.2[c]	100	100	100	100	100
pH 9.6[c]	0	0	0	0	0

[a] Test performed using API 20E, 25°C, 24h
[b] Test performed using API 50CH-E, 25°C, 48h
[c] Test performed at 25°C

3.3.2 *Morganella morganii*

M. morganii is probably the most important and best-known microorganism with respect to histamine formation in seafood (Table 3.2). In agreement with this, *M. morganii* has been identified as the bacteria responsible for histamine formation in three reported outbreaks of HFP (Table 3.1). Two subspecies of *M. morganii* are identified; *M. morganii* subsp. *morganii* and *M. morganii* subsp. *sibonii.* Histamine in toxic concentrations is produced by both the subspecies when tested at 10°C (Paper 3). In contrast to other species, most isolated strains of *M. morganii* decarboxylate histidine (Frank *et al*. 1985; López-Sabater *et al*. 1994a; López-Sabater *et al*. 1996a; Wauters *et al.* 2004).

In fact, very few non-histamine-producing strains have been isolated (Taylor *et al.* 1978). *M. morganii* grows at between 4 - 45°C (Janda and Abbott 2005); however, histamine is not formed in toxic concentrations below ~7°C (Behling and Taylor 1982; Lorca *et al*. 2001; Kim *et al*. 2002; Veciana-Nogués *et al*. 2004). As a consequence, *M. morganii* will not be problematic with respect to histamine formation in seafood stored at 2°C.

3.3.3 *Photobacterium phosphoreum*

P. phosphoreum is a psychrotolerant bacterium capable of histamine formation in toxic doses. As can been seen in Table 3.1, *P. phosphoreum* has been involved in four reported outbreaks of HFP. *P. phosphoreum* is a well-known spoilage bacteria in fish and seafood products (Dalgaard 2006), but it has received little attention as a histamine producer and in many studies it is entirely ignored. However, during the 1980's, significant histamine production by *P. phosphoreum* (N-group bacteria) was documented at low temperatures (Okuzumi *et al*. 1981; Okuzumi *et al.* 1982; Okuzumi *et al*. 1984c; Morii *et al*. 1986; Morii *et al.* 1988). In 2004, *P. phosphoreum* was for the first time isolated and identified in relation to an outbreak of HFP (Table 3.1). *P. phosphoreum* does not grow without salt and at temperatures above 25-30°C. It is also very sensitive to heat. These circumstances might have been the reason why *P. phosphoreum* was not isolated and identified from seafood causing HFP before 2004 (Kanki *et al.* 2004). It seems possible that the *P. phosphoreum* should be divided into subgroups according to the difference in histamine production capabilities (Dalgaard *et al.* 2006).

3.4 Detection of histamine-producing bacteria

Both *M. morganii* and *M. psychrotolerans* grows with atypical colonies in violet-red bile agar when compared to e.g. *E. aerogenes* and *K. oxytoca* (Figure 3.3). The colonies of *Morganella* spp. are small and without precipitation. This might impede their recognition during routine analysis.

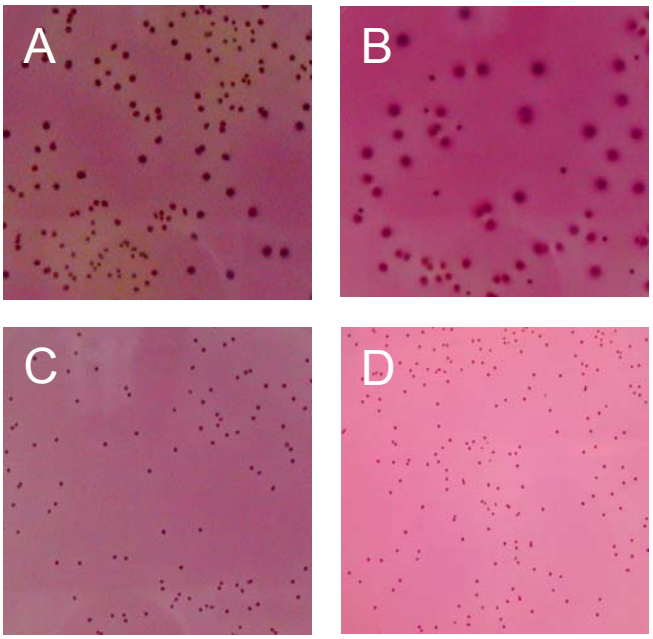


Figure 3.3 Colonies of A) *Enterobacter aerogenes*, B) *Klebsiella oxytoca*, C) *Morganella morganii* and D) *M. psychrotolerans* on tryptic soyagar-violet red bile glucose agar incubated at 25°C at 24 hours. (Unpublished data).

3.4.1 Culture based techniques

Methods based on differential growth-media have been developed to detect decarboxylating bacteria in food. Most of these media use the change in pH that occurs when amino acids are decarboxylated into the more alkaline biogenic amines. Marcobal *et al*. (2006) describes in a review how the first pH-based medium was developed in 1954. This medium is still used for the identification of Enterobacteriaceae (Barrow and Feltham 1999). In 1981, the first solid medium for detection of HPB was published (Niven *et al*. 1981). Niven's medium has been subjected to some criticism due to the presence of false positive results and a low pH-value (pH 5.3) that may inhibit growth of some bacteria. The false positives are typically a result of the production of by-products such as ammonia. Niven's medium has in several experiments been used to identify HPB from fish and seafood products (Subburaj *et al*. 1984; Silva *et al.* 1998; Ben Gigirey *et al*. 1999; Kim *et al*. 2001b). Without success several attempts to improve Niven's medium have been carried out (Yoshinaga and Frank 1982; Smith *et al*. 1982; Mavromatis and Quantick 2002b; Tsai *et al*. 2004).

Methods based on the development of CO_2 have been tested (Marcobal *et al.* 2006). Some are very time- and labour consuming while other can result in false positives since the production of CO_2 can be a result of other reactions than the decarboxylase reaction. Klausen and Huss (1987a) developed a relatively rapid method (25 hours at 25°C) based on the change in conductance that occurs when histidine is decarboxylated into histamine; however, the use of specialised and expensive equipment limits its usefulness.

Many of these detection methods will not detect *M. psychrotolerans* since they involve incubation temperatures at 35-37°C (Niven *et al.* 1981; Taylor and Woychik 1982; Sumner and Taylor 1989; Mavromatis and Quantick 2002b). Methods that allow both mesophilic and psychrotolerant HPB to be detected are therefore needed.

An often-used technique for identification of HPB is to allow the isolates to grow in a histidine-containing broth followed by detection of histamine in the broth (Taylor *et al.* 1978). In many studies, these tests have been performed at 25-37°C (Yoshinaga and Frank 1982; López-Sabater *et al.* 1994a; Masson *et al*. 1996; López-Sabater *et al.* 1996a; Ben Gigirey *et al*. 2000; Kim *et al.* 2000; Petäjä *et al*. 2000). The information obtained at these high temperatures is of little relevance to refrigerated foods (e.g. seafood, meat and dairy products). For chilled seafood, tests at 10°C or below are more appropriate (Ababouch *et al*. 1991b; Paper 1; Paper 2).

3.4.2 Molecular methods

Various PCR methods to detect the gene encoding histidine decarboxylase (*hdc*) have been developed (Landete *et al*. 2007). Primer sets for the detection of *hdc* in both Gram-negative and Gram-positive bacteria are available. Also a PCR assay to detect the four most important decarboxylase genes (histidine, tyramine, putrescine and cadaverine) from a wide range of Gram-positive and Gram-negative bacteria associated to food has been suggested (de las Rivas *et al*. 2006a). The ability of this multiplex PCR method to detect *M. psychrotolerans* has not been studied. The ability of PCR methods to differentiate between weakly and strongly histamine producing bacteria deserves further study. This is important if the PCR methods are to be used in seafood inspection as detection of weak HPB might lead to unnecessary concerns.

PCR methods targeting species-specific sequences rather than *hdc* could avert the detection of weak histamine-producing species. There is, however, the unsolved

problem with the variability in histamine-producing abilities observed in e.g. *P. phosphoreum* (Dalgaard *et al.* 2006). To solve this problem and to be able to distinguish between weak and strongly histamine-producing *P. phosphoreum,* further studies are needed. Kim *et al.* (2003a) developed a PCR assay with 16S rDNA primers. The unique primers for *M. morganii* made it possible to detect levels of 10^6-10^8 CFU/ml in a albacore -homogenate. An enrichment step at 37°C for 6 hours made it possible to detect 9 CFU/ml. However, the enrichment step changes the method from quantitative to qualitative. The method is not suitable for *M. psychrotolerans* due to the high temperature enrichment step. Furthermore, a method that detects the HPB at much lower levels without the enrichment step is needed.

3.5 Occurrence of histamine-producing bacteria

HPB are part of the natural microflora in sea-water and a part of the natural flora in the intestines, gills and on the skin of fresh fish (Corlett *et al*. 1978; Okuzumi *et al.* 1981; Smith *et al.* 1982; Okuzumi and Awano 1983; Okuzumi *et al*. 1984b; Okuzumi *et al.* 1984c; Kim *et al.* 2001b). HPB invade fish flesh from these reservoirs. This is reflected by higher histamine concentrations in fish flesh adjacent gills and intestines and higher histamine concentrations in undressed as compared to dressed fish (Frank *et al*. 1981; Taylor and Speckhard 1983; López-Sabater *et al.* 1996b; Kim *et al*. 1999; Kim *et al.* 2001b). HPB are also found in the water, baskets and floors/equipment at fish processing plants and fish markets (Corlett *et al.* 1978; Subburaj *et al.* 1984).

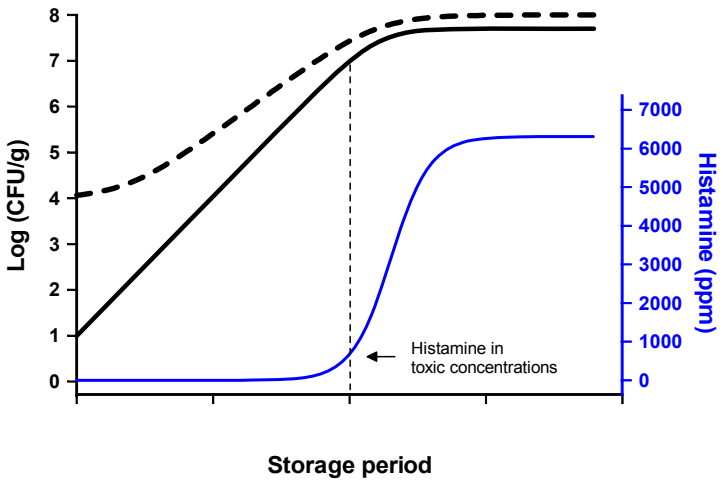


Figure 3.4 Specific spoilage organism (SSO) concept applied to histamine-producing bacteria. Typical changes in aerobic plate counts (dashed line) and histamine-producing bacteria (solid black line) and the concentration of histamine (solid blue line) during storage of fresh fish and seafood. Modified from Dalgaard (2006).

Only about 1% of the surface microflora of fresh fish represents HPB (Kimata 1961; Ababouch *et al.* 1991a; Kim *et al.* 2001b). This agrees to the findings in Paper 1, where bacteria without the ability to produce histamine dominated the microflora of skin, gills, intestines and cut steaks of freshly caught tuna.

The arobic plate count of newly cut tuna steaks can be relatively high (Paper 1). However cold storage and vacuum packaging may inhibit the growth of most of these bacteria allowing growth of the more psychrotolerant and anaerobic HPB e.g. *M. psychrotolerans* and *P. phosphoreum*. As they grow faster than the remaining part of the microflora they become the dominating bacteria. This phenomenon is known as the specific spoilage organism (SSO) concept (Figure 3.4). When the bacterial population of prolific HBP reaches about 10^6 CFU/g histamine can be formed in toxic concentrations (Omura *et al*. 1978; Yoshinaga and Frank 1982).

Okuzumi *et al.* (1984b) observed seasonal variation in the identified HBP from common mackerel. *M. morganii* and the N-group bacteria (later identified as *P. phosphoreum* by Fujii *et al.* (1997)) were both present in the summer samples, whereas the N-group bacteria were the only HPB present in the winter samples. In the same study, storage temperature was also shown to be a selective factor of HPB.

4. Factors affecting histamine formation

The formation of histamine in seafood is obviously influenced by availability of substrate (free histidine) in fish flesh and thereby the fish species. Also 10^6-10^7 HPB/g is needed before histamine can be produced in toxic concentrations (Ryser *et al.* 1984; Tabor and Tabor 1985; Kim *et al.* 2000; Lorca *et al.* 2001; Kim *et al*. 2001a; Guizani *et al*. 2005; Paper 1; Paper 5). Concentrations of HPB are low in the flesh of newly caught fish and significant growth is required before these bacteria to reach levels where histamine in toxic concentrations can be produced (Figure 3.4). Growth and the concomitant formation of histamine by HFB in seafood depend on several environmental factors including temperature, NaCl-concentration, pH, antimicrobial agents and composition of the storage atmosphere (Taylor 1986; Lehane and Olley 2000; Paper 5). To facilitate the control of HFP it is important to know the effect of these parameters on the most important HPB. Factors that influence the rate of histamine formation in seafood are discussed below.

4.1 Microbial contamination

HPB are part of the natural microflora on fresh fish (section 3.5) and cross contamination during handling on board fishing vessels, particularly gutting and cleaning, and during processing is most likely. Gingerich *et al*. (2001) found that HPB did not comprise a large part of the microflora associated with fish-processing facilities. However, for HPB little information is available on routes of contamination during seafood processing. For *M. psychrotolerans* information is needed on routes of seafood contamination as well as on its occurrence in both seafood and the environment.

4.2 Time and temperature

Temperature has a major impact on the formation of histamine in fresh natural contaminated seafood. As part of this thesis information from 124 storage trials has been compiled (Paper 5). These studies indicate that 500 ppm histamine can be formed in naturally contaminated seafood within a few hours at above +16°C, after about two days at +11°C to +15°C, after about three days at +6°C to +11°C and after as little as four but up to more then 25 days at -1°C to +5°C (Paper 5). The marked effect of temperatrure on histamine formation is due to microbial growth and activity and this has been confirmed for fish and broth inoculated with HPB (Figure 3.1; (Arnold *et al.* 1980; Eitenmiller *et al*. 1981; Behling and Taylor 1982; Ryser *et al.* 1984; Tabor and Tabor 1985; Klausen and Huss 1987b; Ababouch *et al.* 1991b; López-Sabater *et al.* 1996b; Kim *et al.* 2000; Paper 5). Clearly, chilling at 0°C (e.g. in ice) or below (e.g. in refrigerated seawater or slush ice) is efficient ways of delaying histamine formation in fresh fish. However, studies of naturally contaminated fresh fish has shown that histamine can be formed at 0°C and that toxic concentration of histamine above 500-1,000 ppm may be formed. This, however, rarely occurs in less than 2-3 weeks (Papar 5; López-Sabater *et al.* 1996b; Dalgaard et al. 2006). It is also important that toxic concentrations of histamine formation in naturally contaminated fresh fish 2-5°C has been observed after about one week of storage although in most studies longer time of storage are required (Paper 1; Papar 5; Ababouch *et al.* 1991a; Dalgaard et al. 2006). The histamine formation in naturally contaminated fresh fish at 0-5°C can be explained by growth and activity of psychrotolerant bacteria including *M. psychrotolerans* and *P. phosphoreum*. This will be discussed in chapter 5 and 6, as it seems the most important factor to reduce the occurrence of HFP (Table 3.1).

Frozen storage during one to several weeks at -20-30°C significantly reduced the concentration of *P. phosphoreum* in fresh fish and markedly delayed histamine formation in the thawed products during chilled storage (Emborg *et al.* 2002; Dalgaard *et al.* 2006). However, concentrations of *E. aerogenes* inoculated in milk and sail fish was not affected by storage at -20°C for 8 weeks and rapid production of histamine was observed in the thawed fish when stored at 25°C (Tsai *et al*. 2005a). The effects of freezing and frozen storage on *M. psychrotolerans* are not known and deserves further study.

4.3 Sodium chloride

Sodium chloride in concentrations above 1-2% reduces the growth rate and delay histamine formation by most Gram-negative HPB (Okuzumi *et al*. 1984a; Yamanaka *et al*. 1985; Ramesh and Venugopal 1986; Yamamoto *et al*. 1991; Ababouch *et al.* 1991b; Wendakoon and Sakaguchi 1993; Morii *et al*. 1994; Aytac *et al*. 2000). The growth rate of *M. psychrotolerans* is clearly affected by the concentration of NaCl in the growth medium (Figure 4.1; Paper 2; Paper 4). In addition the concentration of NaCl also affects the maximum population density (N_{max}). Despite a 10-fold reduction in N_{max} with increasing saltconcentration a corresponding reduction in the histamine concentration was not observed (Paper 4). Similar observations have been obtained with *M. morganii* by Yamamoto *et al*. (1991) and Aytac *et al.* (2000).

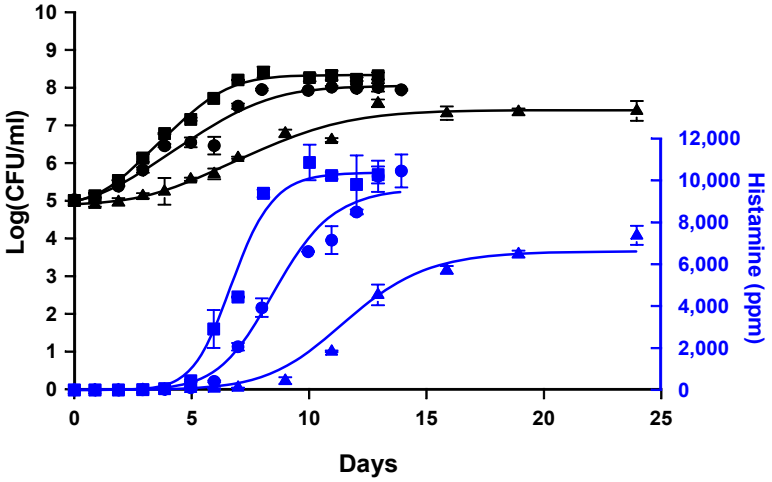


Figure 4.1 Growth (black curves) and histamine formation (blue curves) by *Morganella psychrotolerans* in LB Miller broth at 5°C, pH 5.9 with 2 (■), 3 (●) and 4% (▲) NaCl (Paper 4). Error bars represent standard deviations of duplicate determinations.

Three incidents of HFP caused by cold-smoked tuna containing 1.3% and 2.2% and 4.6% salt in the water phase (WPS) have been reported (Emborg *et al.* 2006). However, products of this type are most often being produced with far higher concentrations of WPS (4.1-12.7%, Paper 2). To prevent toxic histamine formation by *M, psychrotolerans* is has been suggested that cold-smoked tuna should contain 5% WPS or more and that the declared shelf-life of no more than 3 to 4 weeks at 5°C (Paper 2). 5% WPS would also be beneficial for suppressing microbial pathogens (*Clostridium botulinum* and *Listeria monocytogenes* (Paper 2). In New Zealand, hot-

smoked kahawai (*Arripis trutta*) has caused numerous outbreaks of HFP (Bremer *et al*. 2003) and this product has been reported to contain 1.2% NaCl (corresponding to 1.6% WPS). Thus, low salt concentration in smoked fish seems to be an important risk factor for HFP.

4.4 pH

pH of fresh fish and seafood products vary with the species of fish (from 5.4 to 6.5), the season of the year and the type of product (Huss 1995). In yellowfin tuna (*Thunnus albacares*) and in cold smoked tuna pH of 5.8 and 5.8-6.1 respectively are observed (Paper 1; Paper 2).

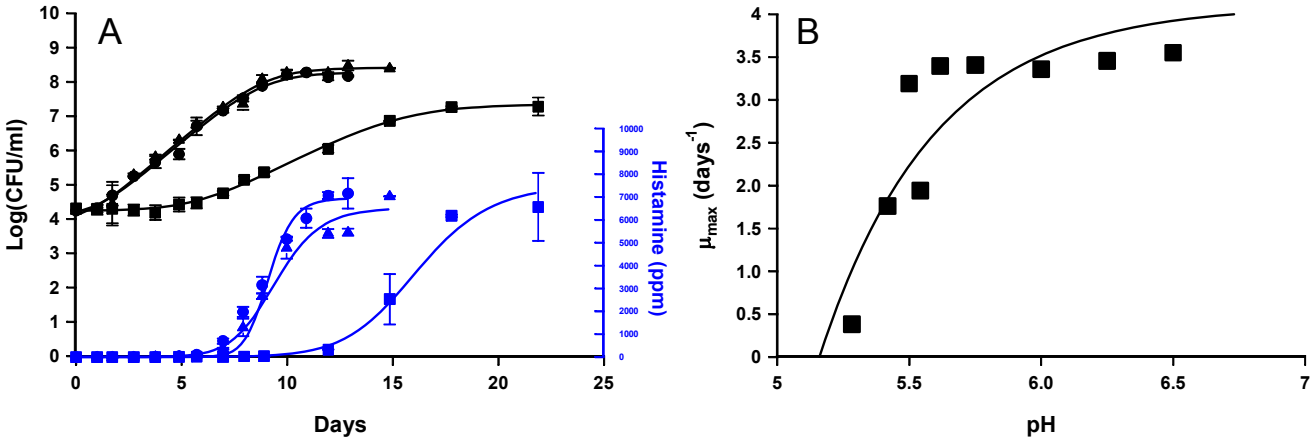


Figure 4.2 A) growth (black curves) and histamine production (blue curves) by *M. psychrotolerans* in LB Miller broth at 5°C with 3% NaCl and at pH 5.6 (■), 5.9 (●) and 6.3 (▲) and B) growth rates (days^{-1}) of *Morganella psychrotolerans* at different pH-values determined in LB Miller broth at 10°C with 1% NaCl. Error bars represent standard deviation from duplicate determinations (Paper 4).

The growth rate of *M. psychrotolerans* was shown to be strongly affected by changes in pH between 5 and 5.6 (Figure 4.2A). However between 5.6 and 6.5 little effect was observed (Paper 4). The decrease of the growth rate prolonged as previously seen the time until histamine was formed in toxic concentrations (Figure 4.2B). pH also seems to lower N_{max} without affecting the histamine concentration (Figure 4.2B), as observed for the concentration of NaCl (Figure 4.1). These findings are in agreement to what was earlier observed for *M. morganii* (Kimata 1961; Eitenmiller *et al.* 1981).

4.5 Atmosphere

Seafood packed in modified atmosphere can be distributed through retail cabinets in supermarkets. Another advantage of modified atmosphere packaging (MAP) is that growth and metabolite production of the spoilage bacteria is delayed, and shelf-life therefore extended. The shelf-life extension observed with seafood, however, is not as pronounced as observed e.g. with fresh meat (Huss 1995). Studies has shown that a MAP with gas mixtures containing carbon dioxide (CO_2) and nitrogen (N_2) have minor effect on the HPB present in fresh seafood when compared to storage in air, while an O_2 containing atmospheres decrease the growth rate of HBP and thereby increases the safe shelf-life (Paper 1; Paper 4). Likewise it was shown that the activity of inducible HDC isolated from *P. phosphoreum* decreased with increasing oxygen tension (Morii *et al.* 1994; Morii and Kasama 1995).

For *M. psychrotolerans* increasing concentrations of CO_2 decreased the maximum polulation density (N_{max}) (as observed for NaCl and pH) but did not affect the maximum concentration of histamine produced (Paper 4). Furthermore it was shown that an O_2-containing atmosphere lowered the growth rates significantly compared to vacuum packaging (VP) (Paper 1). This is previously observed for *M. morganii* (Aytac *et al.* 2000). VP is widely used for fresh tuna however, and precisely VP tuna has caused several incidents of HFP in England and Wales. To reduce the problems with HFP due to VP tuna it was suggested, that modified atmosphere packaging containing 40% CO_2 and 60% O_2 were used instead of VP (Paper 1).

Filtered wood smoke contains moderate concentrations of carbonmonooxide (CO) and CO_2 and is used for packaging of fresh fish in the USA. It has been shown that the treatment decreases the growth rate and delay the formation of histamine in mahi-mahi fillets compared to untreated fillets in air (Kristinsson *et al*. 2007). However the use of filtered wood smoke is not allowed within the EU (Directive 95/2/EC on food additives other than colours and sweeterners) due to the presence of CO. CO act as a colour stabilising component and may mask visual signs of spoilage to the consumer (Smulevich *et al*. 2007).

4.6 Other preserving parameters

Alternative preserving compounds like clove and cinnamon was shown to have a significant inhibitory effect on growth and histamine formation by *M. morganii* and *E. aerogenes* when 1% was applied to inoculated broth. The addition of NaCl enhanced the effect of clove (Wendakoon and Sakaguchi 1993; Shakila *et al*. 1996). Cardamom and turmeric exhibited a moderate inhibitory effect whereas pepper was ineffective (Shakila *et al.* 1996). Likewise, it was shown, that the use of potassium sorbate at a concentration of 0.5% inhibited growth and histamine foramtion by *M. morganii* and *Klebsiella pneumoniae* for at least 120 hours at 32°C and for 216 hours at 10°C. Sodium hexametaphosphate and sodium polyphosphate was less effective against these HPB (Taylor and Speckhard 1984). A gamma irradiation dose of 2.0 kGy decreased the concentration of *M. morganii* significantly compared to 0.5 kGy, and delayed ihistamine formation (Aytac *et al.* 2000). For *M. psychrotolerans,* the effect of spicies, organic acids and other preserving parameters remain to be determined.

5. Modelling growth and histamine formation by *Morganella psychrotolerans*

Mathematical models to predict the effect of environmental parameters on the growth of HPB and on their formation of histamine could be of considerable practical importance to the seafood sector and inspecting authorities. A predictive model can for example be used for:

- Estimation of shelf-life so formation of histamine in high concentrations in seafood under realistic storage conditions is prevented.
- Optimisation of storage conditions so a desired shelf-life is obtained.
- Exposure assessment studies where the concentration of histamine in seafood must be determined at the time of ingestion. In fact, more detailed exposure assessment studies cannot be carried out unless models are available to predict histamine formation in seafood over time and as a function of product characteristics and storage conditions.

Despite the high occurrence of HFP, limited research concerning prediction of histamine formation in fresh and lightly preserved seafood products have been performed. Several parameters influence the formation of histamine in seafood (see Chapter 5) and the most important parameters must be included in the predictive models. Once developed the models must be validated, to ensure that predictions are applicable to observed measurements during processing and storage of naturally contaminated products. Validated mathematical models that accurately predict histamine formation in seafood are important as they can contribute to a reduction of histamine intake by consumers.

In this chapter development and validation of mathematical models for growth and histamine formation of *M. psychrotolerans* will be discussed and compared to the few existing models for histamine formation in food.

Growth of microorganisms in food typically follows a pattern with four stages referred to as the lag-, exponential-, stationary- and the death phase. The death phase

is typically observed when seafood products are sensory spoiled thus, not important for HFP and is not included in the present thesis. During the lag-phase cells are adjusting their physiology to environmental changes (e.g. packaging, addition of NaCl, change in temperature etc.) and no increase in cell concentrations is observed (Figure 5.1). The length of the lag-phase varies dependent on a magnitude of the environmental changes, and the physiological state of the microorganism. If growing cells are transferred from one environment to a similar one, a lag-phase may not occur (Ross and Dalgaard 2004). During the exponential phase, cells multiply by a constant rate referred to as the maximum specific growth rate (μ_{max}) (Figure 5.1).

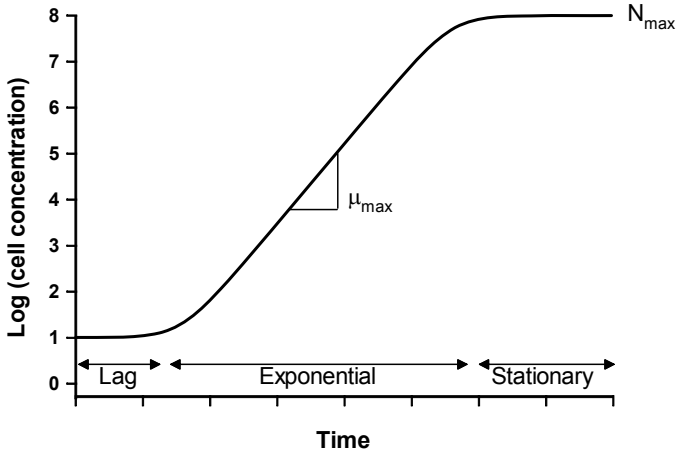


Figure 5.1 A typical microbial growth curve showing the lag, exponential and stationary phase. μ_{max} is the maximum specific growth rate and N_{max} the maximum population density.

μ_{max} is affected by environmental parameters. Depletion of nutrients or accumulation of metabolites will also at some stage reduce the specific growth rate and lead to the stationary phase where the microorganism reach their maximum population density (N_{max}) (Figure 5.1). Changes in cell density over time (Figure 5.1) can be described with primary growth models to estimate kinetic parameters including lag time, μ_{max} and N_{max} (McMeekin *et al*. 1993; McKellar 2004). Secondary models can then be used to describe how environmental conditions like temperature, atmosphere, pH, water activity (a_w) etc. influence the kinetic parameters (Whiting 1995; Ross and Dalgaard 2004).

Formation of metabolites can be modelled by use of the yield concept. The yield concept suggests that the formation of biomass can be related to consumption of substrate by a constant yield factor (Monod 1949; Bailey and Ollis 1986). In a similar way the yield concept can be used to relate microbial metabolite formation to growth (Luedeking and Piret 1959; Jørgensen *et al*. 2000b; Dalgaard 2002). The yield concept has previously been used for modelling of histamine and tyramine formation in cold smoked salmon (Jørgensen *et al.* 2000b) and for trimethylamine formation in cod by *P. phosphoreum* (Dalgaard 1995b) Furthermore, histamine formation by *M. morganii* in fishmeal (Torres *et al*. 2002) and in jack mackerel (Bermejo *et al*. 2004) have been modelled using the yield concept.

5.1 Growth of *Morganella psychrotolerans*

5.1.1 Primary modelling

Primary growth models should appropriately describe the kinetics of growth with as few parameters as possible. Numerous growth models have been developed (see reviews by Baranyi and Roberts (1994); Skinner *et al*. (1994); Whiting (1995); Baranyi and Roberts (1995); McDonald and Sun (1999); McKellar (2004)). The so-called Baranyi model is popular but complicated and most useful when included in application software (Food Combase Predictor (http://www.combase.cc/predictor.html), MicroFit (http://www.combase. cc/predictor.html) and DMfit (http://www.ifr.ac.uk/safety/DMFit/)).

In the present thesis an integrated and log transformed version of the expanded Logistic model (Turner *et al*. 1969; Dalgaard 2002) was used as primary model to describe growth by *M. psychrotolerans* (Eqn. 1). This model includes a lag phase and it is more flexible than the classical Logistic model where the parameter m has a fixed value of 1.0. The parameter m controls the degree of growth dampening when the concentration of cells (N) approaches the maximum population density (N_{max}). An example is shown in Figure 5.2 for m = 1 and m = 0.4.

$$Log\ N_t = Log\ N_0 \qquad t < t_{lag}$$

$$Log\ N_t = Log\left(\frac{N_{max}}{\left(1+\left(\left(\left(\frac{N_{max}}{N_0}\right)^m - 1\right)\cdot e^{(-\mu_{max}\cdot m\cdot(t-t_{lag}))}\right)\right)^{1/m}}\right) \qquad t \geq t_{lag} \qquad \textbf{Eqn. 1}$$

In Eqn. 1 N_t and N_0, (CFU/g) are the concentrations of *M. psychrotolerans* at time *t* and 0, respectively. μ_{max} is the maximum specific growth rate, *m* is the dampening parameter that influence growth when N_t approaches N_{max}. *t* is storage time (h) and t_{lag} the lag time (h).

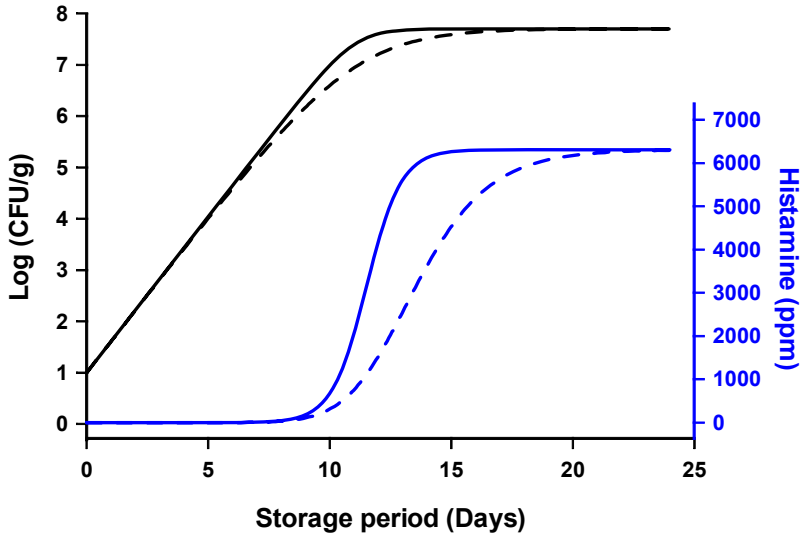


Figure 5.2 Predicted growth (black lines, log CFU/g) and histamine formation (blue lines, ppm). Growth and histamine formation was simulated by the expanded Logistic model (Eqn. 1) with *m* = 1.0 (solid lines) and *m* = 0.4 (dashed lines). For each of the growth curves histamine formation was predicted by using a constant yield factor ($Y_{His/CFU}$) of 0.000000063 mg histamine/CFU.

By combining the expanded logistic growth model with a yield factor ($Y_{his/CFU}$, mg/CFU) a primary model for histamine formation was obtained (see section 5.2).

In the present thesis the concept of relative lag time (RLT, Eqn. 2) is used (Ross (Ross 1999; Mellefont *et al*. 2003). The concept is based on the hypothesis that the lag time is determined by the amount of work a microorganism has to do to adapt to a new environment and the rate at which this work can be done. RLT is calculated as the ratio of lag time (t_{lag}) and generation time (t_g) at identical conditions (Eqn. 2).

$$RLT = \frac{t_{lag}}{t_g} = \frac{t_{lag} \cdot \mu_{\max}}{\ln(2)}, \qquad t_{lag} = \frac{RTL \cdot \ln(2)}{\mu_{\max}} \qquad \textbf{Eqn. 2}$$

5.1.2 Secondary modelling

Secondary models to predict the effect of environmental conditions on the kinetic parameters used in the primary model, have been extensively reviewed (Skinner *et al*. 1994; Whiting 1995; McDonald and Sun 1999; Ross and Dalgaard 2004). Several types of secondary models are widely used including square-root type models, cardinal parameter models, Arrhenius-type models, polynomial models and artificial neural network (ANN) models.

Square root type models (Eqn. 3) were initially suggested to replace Arrhenius models for the effect of temperature growth rates of bacteria (Ratkowsky *et al*. 1982).

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \qquad \textbf{Eqn. 3}$$

In eqn. 3 *b* is a constant and *T* is the storage temperature. The parameter T_{min} is the theoretical minimum temperature for growth (Figure 5.3). Importantly T_{min} is a parameter that characterises the effect of temperature on growth of a microorganism. Its estimated value is below the lowest temperature at which growth of a microorganism is actually observed as shown in Figure 5.3 for *M. psychrotolerans*. In expanded square-root type models parameters similar to T_{min} are used to characterise the effect of e.g. pH (pH_{min}), a_w ($a_{w\ min}$) and the CO_2 concentration in modified atmospheres ($CO_{2\ max}$) (Ross and Dalgaard 2004).

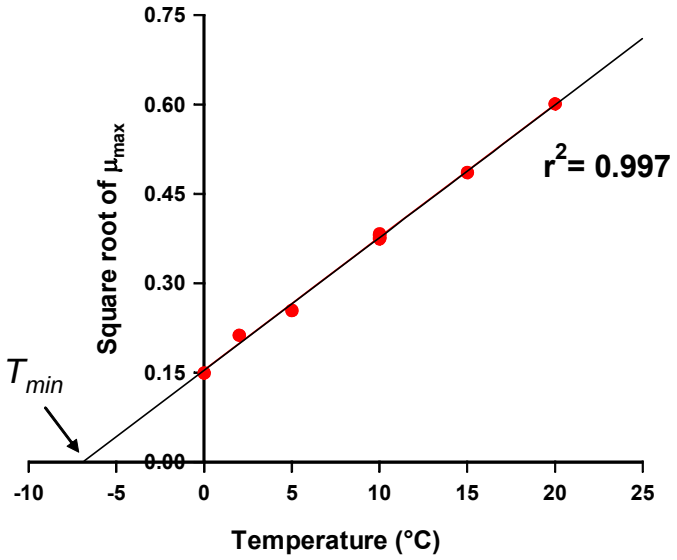


Figure 5.3 Square-root transformed growth rates plotted against the temperature at which they were obtained. T_{min} can be determined as the intercept between the model and the temperature axis.

Within predictive food microbiology, cardinal parameter models (CPM) were described by Rosso *et al* (1995). These model are in several ways similar to to the square-root type model and they both include parameters with a biological interpretation. However, CPM includes parameters for minimum, maximum and optimum growth conditions (e.g. T_{min}, T_{opt}, T_{max}, pH_{min}, pH_{opt}, pH_{max}). CPM are also related to the so-called gamma (γ)-concept. This concept was introduced by Zwietering *et al.* (1992) and based on the assumption that: (i) environmental parameters act independently on the growth rate of microorganisms and (ii) the combined effect can be predicted simply by multiplying terms for contributions from different parameter (Eqn. 4.) Thus, dimensionless gamma-factors (γ(i)) describe the decrease in growth rate under sub-optimal conditions (Eqn. 5).

$$\mu_{\max} = \mu_{\max\ opt} \cdot \left(\gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \cdot \gamma(CO_2) \cdot \ldots \gamma(other)\right) \qquad \textbf{Eqn. 4}$$

$$\gamma = \frac{Growth\ rate\ at\ actual\ condition}{Growth\ rate\ at\ optimal\ condition}, \qquad \gamma(T) = \frac{T - T_{\min}}{T_{opt} - T_{\min}} \qquad \textbf{Eqn. 5}$$

In eqn. 4 and 5 $\mu_{max\ opt}$ is the maximum specific growth rate at optimum conditions and γ(i) the relative effect of the different parameters. Importantly, each gamma-factor (γ(i)) must have a value between 0 and 1.

Square-root type models like CPM can be formulated so that terms for environmental parameters have values between 0 and 1 (Ross and Dalgaard, 2004, Eqn. 6; Eqn. 7). The main different between square-root type models and CPM is then the reference temperature that can be selected for the former but must be the optimum growth temperature for the latter. This difference can be important for psychrotolerant bacteria like *M. psychrotolerans* where growth at high temperature has little relevance for their importance in seafood.

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \leftrightarrow \mu_{\max} = b \cdot (T - T_{\min})^2 \leftrightarrow \mu_{\max} = b' \cdot \left(\frac{T - T_{\min}}{T_{ref} - T_{\min}} \right)^2 \qquad \textbf{Eqn. 6}$$

In eqn. 6, T_{ref} is a reference temperature which is lower than the temperature optimum for growth. The constant *b'* correspond to μ_{max} at T_{ref}.

In the present thesis Eqn 7, was used to model the effect of environmental parameters (temperature (T), CO_2, pH and a_w) on square-root transformed growth rates (μ_{max}) of *M. psychrotolerans* (Paper 4).

$$\mu_{max} = b \cdot \left(\frac{T - T_{\min}}{T_{ref} - T_{\min}} \right)^2 \cdot \frac{a_w - a_{w\min}}{A_{w\,opt} - a_{w\min}} \cdot 1 - 10^{(pH\,\min - pH)} \cdot \left(\frac{CO_{2\max} - CO_2}{CO_{2\max} - CO_{2\,opt}} \right)^2 \cdot \xi \qquad \textbf{Eqn. 7}$$

In eqn. 7 *b* corresponds to μ_{max} at the reference temperature (T_{ref} = 25°C) whereas T_{min}, $a_{w\ opt}$, $a_{w\ min}$, pH_{min}, $CO_{2\ opt}$ and $CO_{2\ max}$ are the theoretical cardinal parameters

describing the effect of temperature, a_w, pH and CO_2 on μ_{max}. For *M. psychrotolerans* $CO_{2\ opt}$ and $a_{w\ opt}$ was assumed to be 0 and 1, respectively (Paper 4).

A term (ξ) was calculated as described by Le Marc *et al.* (2002) to take into account the effect of the interaction between the environmental parameters on μ_{max} (Eqn. 7). Interaction between environmental parameters can be important and this has for example been shown for *Listeria monocytogenes* when growth in lightly preserved seafood is influenced by temperature, a_w, pH, lactic acid, phenol and diacetate (Mejlholm and Dalgaard 2007b).

Table 5.1 Cardinal parameter values for some microorganisms associated to seafood (modified from Dalgaard (2002)).

	Parameter values				
Organism	T_{min}	$a_{w\ min}$	pH_{min}	$\%CO_{2\ max}$	References
Shewanella putrefaciens	-8.0 to -9.0	0.95	Nd[a]	150-156	Dalgaard (1993); Dalgaard (1995a); Koutsoumanis *et al*. (2000)
Pseudomonads	-6.1 to -11.4	0.95	nd	121	Neumeyer *et al*. (1997); Koutsoumanis *et al.* (2000)
Photobacterium phosphoreum	-9.0	0.95	4.3	376	Dalgaard (1993); Dalgaard *et al*. (1997)
Lactic acid bacteria	-3.1 to -11.4	0.93	4.2	232	Koutsoumanis *et al.* (2000); Dalgaard *et al*. (2004), Mejlholm and Dalgaard (2007a)
Brochothrix thermosphacta	-10.9	nd	Nd	187	Koutsoumanis *et al.* (2000)
Listeria monocytogenes	-2.3	0.92	5.0	112	Mejlholm and Dalgaard (2006)
Enterobacteriaceae	-0.7	nd	nd	nd	Dalgaard *et al.* (2004)
Morganella psychrotolerans	**-5.8**	**0.96**	**5.1**	**273**	Paper 4

[a] Not determined

The developed growth rate model (Eqn. 7) for *M. psychrotolerans* showed this bacterium to be slightly more sentive towards chilling than *Shewanella* and *P. phosphoreum*, but similar to psychrotolerant pseudomonades (Table 5.1). Psychrotolerant pseudomonades, *Shewanella* and *P. phosphoreum* are typical spoilage bacteria in iced fresh fish. However, *M. psychrotolerans* is more resistant to CO_2 than *Shewanella* and psychrotolerant pseudomonades but less tolerant towards high concentrations of NaCl (Paper 5). In disagreement to a challenge test (Paper 2) it was found that, *M. psychrotolerans* was more sensitive towards NaCl than *P. phosphoreum*.

Polynomial models have been applied to describe the effect of many different environmental conditions. They are attractive because they relatively easily fits to experimental data by multiple linear regression and they allow most of the environmental parameters and their interactions to be taken into account (Gibson *et al*.

1988; Ross and Dalgaard 2004). However, their usefulness as secondary predictive models has some limitations. Firstly, a large number of parameters without biological interpretation. Secondly, polynomial models with cubic or quadratic terms have been criticised for being too flexible and with a tendency to model experimental errors (Baranyi *et al*. 1996). To overcome the problem with polynomial models being to flexible the use of constrained polynomial models was suggested (Geeraerd *et al*. 2004; Francois *et al*. 2005). In this way, illogical interpolation results and overfitting of the data can be avoided.

A constrained polynomial model was used in the present thesis to model the effect of environmental parameters on log N_{max} for *M. psychrotolerans* (Paper 4) (Eqn. 8).

$$\log N_{\max} = b_0 + b_1 \cdot a_w + b_3 \cdot CO_2 + b_4 \cdot a_w^{\,2} \qquad \textbf{Eqn. 8}$$

The maximum population density of *M. psychrotolerans* was clearly affected by the concentration of NaCl (included in the model as a_w) (Figure 4.1, Paper 4). This phenomenon was explained by elongation of the cells when exposed to salt stress (Figure 5.4). This phenomenon has been observed previously for *Salmonella* and *Listeria* (Mattick *et al*. 2000; Geng *et al*. 2003; Hazeleger *et al*. 2006; Mukhopadhyay *et al*. 2006). With specific staining techniques it has been documented for *Salmonella* and *Listeria* that the prolonged cells or filaments were composed by several normal sized cells (Hazeleger *et al.* 2006).

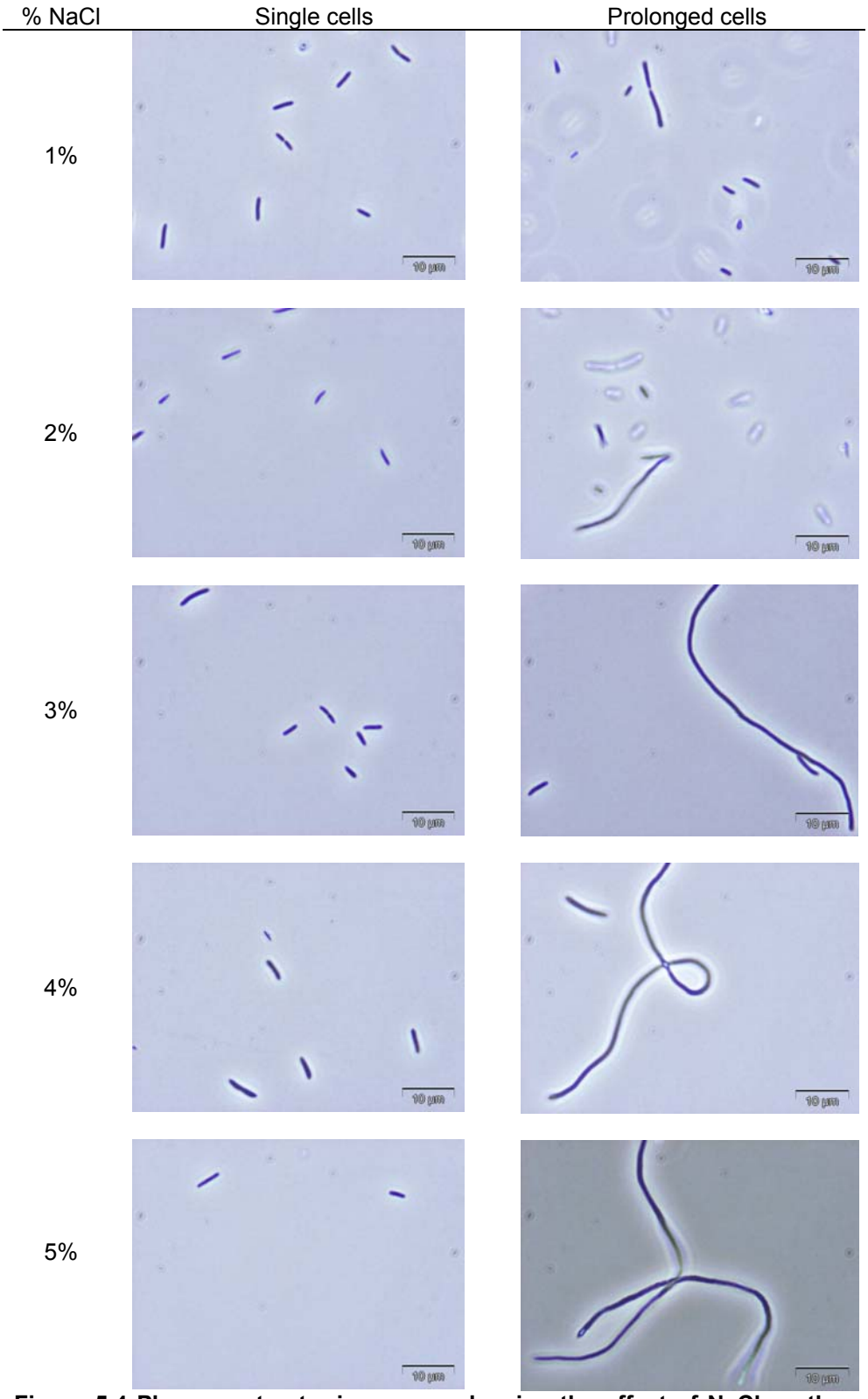


Figure 5.4 Phase contrast microscopy showing the effect of NaCl on the length of *M. psychrotolerans* cells (1000X magnification - Unpublished data).

Various results were obtained with DAPI (4',6-Diamidion-2-phenylindole) staining of *M. psychrotolerans* exposed to high salt (results not shown) and could neither disprove or confirm the hypothesis. If the filaments are composed of several cells it might mean that one prolonged cell result in one single colony while, the histamine formation of the prolonged cell corresponds to the outcome from several cells. This also explains the apparent increase in histamine yield per cell ($Y_{his/CFU}$) observed for high NaCl concentrations. The developed model for histamine formation quantitatively takes this phenomenon into account. It is expected, even though not documented, that the same phenomenon occurs when *M. psychrotolerans* are exposed to increasing CO_2 concentrations.

Experimental data suggested N_{max} of *M. psychrotolerans* was affected by pH (Figure 4.2B). The effect, however, was not statistically significant. This may be due to a limited number of experiments with viable count growth data at different pH. Growth rate-values for different pH were primarily obtained by automatically absorbance measurements (Paper 4). Thus, further studies are needed to determine if pH should be included in the model for N_{max}.

5.2 Histamine formation by *Morganella psychrotolerans*

Under the assumption, histamine formation is unaffected by the growth phases the yield concept was used to relate growth and histamine formation of *M. psychrotolerans* (Eqn. 9, Paper 4).

$$His_t = His_0 + \left(Y_{His/cfu} \cdot \left(N_t - N_0\right)\right) \qquad \textbf{Eqn. 9}$$

In Eqn. 9 His_t and His_0 are the concentrations of histamine (ppm) at time t and 0, respectively. $Y_{His/CFU}$ is the yield factor for histamine formation per CFU (mg histamine/CFU). N_t and N_0 is the cell concentration (CFU/g or CFU/ml).

The yield factor ($Y_{his/CFU}$) was influenced by the environmental parameters but variation in log($Y_{his/CFU}$) was related to variation in log N_{max} (Eqn. 10).

$$\log Y_{His/cfu} = b_0 + b_1 \cdot \log N_{\max} \qquad \textbf{Eqn. 10}$$

The yield factor has previously been used for comparison of the histamine producing abilities for several bacterial strains (Jørgensen *et al.* 2000b). In that study the apparent yield factor was calculated as:

$$pY_{his/CFU} = -\log\left(\frac{His_{final} - His_{initial}}{N_{final} - N_{initial}}\right) \qquad \textbf{Eqn. 11}$$

where $N_{initial}$ and N_{final} are the initial and final cell concentration (CFU/g). $His_{initial}$ and His_{final} are the initial and final concentration of histamine (ppm). Jørgensen *et al*. (2000b) found the highest histamine formation per cell i.e. the lowest $pY_{His/CFU}$ for *P. phosphoreum*.

NaCl- and CO_2-concentrations affected the yield factor for *M. psychrotolerans* and a secondary model to describe these effects was developed (Paper 4). Using this model the yield factor for histamine formation by *M. psychrotolerans* was compared to yield factors for histamine formation by other HPB as different environmental conditions (Table 5.2). In most cases, the predicted yield factor for histamine formation by *M. psychrotolerans* was higher than or similar to values observed for other HPB.

In some experiments with *M. psychrotolerans* an increase in the histamine concentration was observed after the time when the bacteria reached the maximum population density (Figure 3.1; Paper 4). In these situations, a constant yield factor for histamine formation is not optimal to describe the entire histamine formation curve. However, to predict the time to reach toxic histamine concentration (500-100 ppm) the simple yield concept seemed appropriate (Figure 3.1, Figure 4.1, Figure 4.2, Paper 4).

Table 5.2 Apparent yield factor values ($pY_{his/CFU}$) for formation of histamine by selected strongly histamine producing strains and the predicted yield factor value for *Morganella psychrotolerans* stored at the same conditions.

Organism	Substrate	Temp (°C)	Time (hours)	$pY_{his/CFU}$ (-log(µg/CFU))	Predicted $pY_{his/CFU}$ (-log(µg/CFU *Morganella psychrotolerans*)[a]	References
Enterobacter aerogenes	Mackerel extract	20	96	5.3 ± 0.1 (n=2)	4.9	Wendakoon and Sakaguchi (1992)
	Broth	10	168	6.0	4.9	Ryser *et al*. (1984)
	Broth	20	96	5.9	4.9	Ryser *et al*. (1984)
Klebsiella oxytoca	Tuna	8	84	4.3	4.8	López-Sabater *et al*. (1996b)
	Tuna	20	15	4.6	4.9	Veciana-Nogués *et al*. (2004)
	Tuna	20	18	4.8	4.8	López-Sabater *et al*. (1996b)
Klebsiella pneumoniae	Broth	10	48	5.8	4.8	Ryser *et al*. (1984)
	Broth	20	24	5.9	4.8	Ryser *et al*. (1984)
Morganella morganii	Broth	4	336	-[b]	4.6	Kanki *et al*. 2004 (2004)
	Mackerel/albacore/mahi-mahi	4	336	-	4.9	Kim *et al*. (2002)
	Bluefish	15	72	2.8	4.8	Lorca *et al*. (2001)
	Tuna	20	15	3.5	4.9	Veciana-Nogués *et al*. (2004)
	Tuna	8	84	4.1	4.8	López-Sabater *et al*. (1996b)
	Albacore juice	15	72	4.6	4.9	Kim *et al*. (2000)
	Broth	12	240	4.7	4.6	Kanki *et al*. 2004 (2004)
	Tuna	20	18	4.8	4.8	López-Sabater *et al*. (1996b)
	Mackerel extract	20	96	4.9 ± 0.2 (n=2)	4.9	Wendakoon and Sakaguchi (1992)
	Bluefish	10	120	5.1	4.8	Lorca *et al.* (2001)
	Broth	20	120	5.2	4.6	Kanki *et al*. 2004 (2004)
	Broth	25	40	5.3	4.9	Klausen and Huss (1987b)
	Broth	10	168	5.8	4.8	Ryser *et al*. (1984)
	Broth	20	96	5.9	4.8	Ryser *et al*. (1984)
	Mackerel/albacore/mahi-mahi	15	48	6.1 ± 0.1 (n=3)	4.9	Kim *et al*. (2002)
	Bluefish	5	168	6.3	4.8	Lorca *et al*. (2001)
Morganella psychrotolerans	Tuna	2	528	4.1	4.9	Paper 1
	Tuna	2	720	4.3	4.6	Paper 1
	Broth	10		4.5	4.9	Paper 1
Photobacterium phosphoreum	Tuna	2	528	3.4	4.9	Paper 1
	Broth	10		4.2	4.9	Paper 1
	Broth	10		4.4	4.9	Paper 2
	Herring	7	120	4.4 ± 0.3 (n=2)	4.6	Van Spreekens (1987)
	Broth	4	336	4.5	4.6	Kanki *et al*. 2004 (2004)
	Broth	20	120	4.6	4.6	Kanki *et al*. 2004 (2004)
	Broth	12	240	4.6	4.6	Kanki *et al*. 2004 (2004)
	Herring	4	192	5.0 ± 1.4 (n=2)	4.6	Van Spreekens (1987)

[a] Where no information on NaCl concentration and pH where provided, the following values were assumed: pH 5.9, NaCl in broth: 0.5%, in fish, fish juice and extract: 0.3%

[b] No histamine produced.

5.3 Comparison of models for histamine formation in seafood

Prior to the present thesis more detailed mathematical models have not been developed for histamine formation in seafood (Paper 4). However, a few simple models that take into account the effect of storage temperature but not the initial concentration of bacteria have been suggested. These models are briefly described and an evaluation of their performance for prediction of histamine formation in different seafoods is presented.

Frank *et al*. (1983) suggested a simple and entirely empirical model describing the formation of histamine in skipjack tuna when stored at constant temperatures between 70°F and 100°F (21.1°-37.8°C) (Eqn. 12):

$$\text{Histamine (mg/kg)} = 28 \cdot 10^{-32} \cdot \text{Time}^{4.86} \cdot \text{Temp}^{13.85} \qquad \textbf{Eqn. 12}$$

where *Time* is the storage time in hours and *Temp* is the storage temperature in °F. The model was developed on the basis of histamine concentrations in skipjack tuna as determined for 34 different combinations of storage temperature (21.1°C - 37.8°C) and time (0 - 42 h). No other parameters were taken into account. As shown in Paper 5 this simple model provide predictions for the formation of toxic concentration of histamine in skipjack tuna that correspond reasonably with the histamine formation observed in some other studies with this fish species. However, the model is not very accurate for skipjack tuna and for other fish species the lack of accuracy seems to be even worse.

Frank *et al.* (1981) showed the histamine formation in skipjack tuna to be faster in portions of tuna flesh close to the head (anterior part) than in portions of flesh closer to the tail (posterior part). As shown in Figure 5.5 the model of Frank *et al.* (1983) provides predictions corresponding to the highest histamine concentrations observed in anterior parts of skipjack tuna.

By using the same approach as for the model described above Frank and Yoshinaga (1987) developed an empirical model for histamine formation in skipjack tuna stored at constant temperatures between 30°F and 60°F (-1.1°C to +15.6°C) (Eqn. 13):

$$\text{Histamine (mg/kg)} = 19.27 \cdot 10^{-7} \cdot (\text{Temp} - 12.92)^{4.436} \cdot (1.0907)^{\text{Time}} \qquad \textbf{Eqn. 13}$$

where *Temp* is the storage temperature in °F and *Time* is the storage time in days. Unfortunately, the ability of this model to predict histamine formation in skipjack tuna or in other seafoods at low temperature seems limited (Table 5.3).

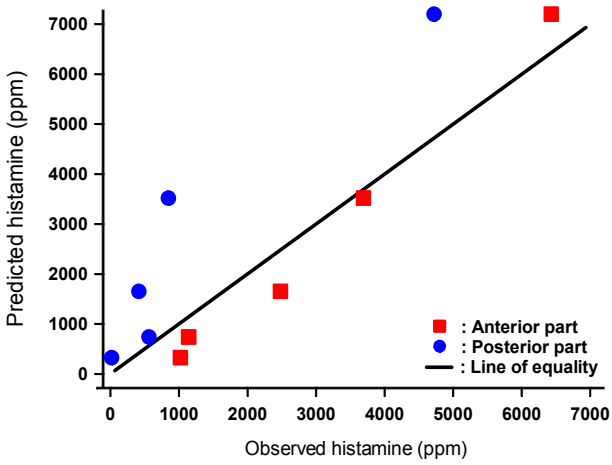


Figure 5.5 Comparisons of histamine concentrations (ppm) predicted by the model of Frank *et al.* (1983) and concentrations observed in the anterior or posterior part of skipjack tuna. The fish was stored for 24 hours at different storage temperatures from 26.7°C to 37.8°C.

Table 5.3 Comparisons of histamine concentrations as observed in storage trials with seafood and concentrations predicted by the model of Frank and Yoshinaga (1987).

Fish species	Temp. (°C)	Time (days)	Histamine (ppm)		Difference (%)[b]	References
			Predicted	Observed		
Tuna	0	21	6	950	-99.4	López-Sabater *et al.* (1996b)
Garfish, spring	0	15	3	21	-83.8	Dalgaard *et al.* (2006)
Garfish, autumn	0	17	4	406	-99.0	Dalgaard *et al*. (2006)
Yellowfin tuna	0	17	4	26	-84.5	Kerr *et al.* (2002)
Yellowfin tuna	0	17	4	77	-94.8	Kerr *et al.* (2002)
Yellowfin tuna	2	19	10	140	-92.6	López-Gálvez *et al.* (1995)
Mackerel	2	12	6	14	-59.7	Fernández-Salguero and Mackie (1979)
Skipjack	4	9	8	2,800[a]	-99.5	Silva *et al*. (1998)
Skipjack	4	12	11	4,000[a]	-99.6	Silva *et al.* (1998)
Yellowfin tuna	4	15	14	61	-77.0	Kerr *et al.* (2002)
Yellowfin tuna	4	15	14	150	-90.6	Kerr *et al*. (2002)
Garfish, spring	5	9	11	893	-98.7	Dalgaard *et al*. (2006)
Garfish, autumn	5	7	9	1,270	-99.3	Dalgaard *et al.* (2006)
Bluefin tuna	8	7	21	506	-95.9	Veciana-Nogués *et al.* (1997)
Bluefin tuna	8	9	24	3,682	-99.3	Veciana-Nogués *et al*. (1997)
Skipjack	10	6	30	6,000[a]	-99.7	Silva *et al.* (1998)
Yellowfin tuna	17	2	77	71	+8.0	Kerr *et al.* (2002)
Yellowfin tuna	17	2	77	150	-48.9	Kerr *et al.* (2002)

[a] The histamine concentrations were measured in the anterior part of the Skipjack tuna.
[b] Calculated as ((predicted-observed)*100/observed).

More recently Torres *et al.* (2002) proposed a model for the effect of temperature (10°C to 30°C) on growth and histamine formation by the mesophilic and histamine-producing bacterium *M. morganii*. The growth and histamine formation by this bacterium was stated by Torres *et al*. (2002) to be independent of pH within the range from 5.5 to 7.0. The effect of temperature on the maximum specific growth rate (μ_{max}) of *M. morganii* was described by Arrhenius kinetics (Eqn. 14).

$$\mu_{\max}(\text{days}^{-1}) = \text{A} \cdot \text{Exp}\left(\frac{-\text{E}_\text{a}}{\text{R} \cdot \text{T}}\right) \qquad \textbf{Eqn. 14}$$

with A = 3.63*10^{16} (days^{-1}), the activation energy (E_a = 88.89 kJ*mol^{-1}) and the gas constant (R = 8.31 J*mol^{-1} * K^{-1}). A specific primary model to describe growth of *M. morganii* as a function of time was not specified by Torres *et al*. (2002) who found a simple yield factor sufficient to relate histamine formation to growth of *M. morganii.* In addition Bermejo *et al.* (2002; 2004) suggested a primary model for histamine formation by mixed microflora broth and fresh fish.

The models by Torres *et al.* (2002) and Bermejo *et al.* (2002; 2004) were developed for Chilean jack mackerel (*Trachurus murphyii*) and jack mackerel (*Trachurus symmetricus)* respectively. Fish species primarily used for reduction to fish meal. The maximum (observed and predicted) concentration of histamine was 200-300 ppm, which is markedly different from products of main interest in the present thesis.

A secondary polynomial model including the effect of temperature, pH and NaCl on growth rate, lagphase and the formation of 2-phenylethylamine, tyramine and total biogenic amines by the Gram-positive *Enterococcus faecalis* in skim milk was proposed by Gardini *et al.* (2001). The growth rate of *E. faecalis* was influenced by all three parameters and it was conclude that the production of amines was mainly dependent on the extent of growth. However, the model for formation of biogenic amines was not related to the growth rate and temperature was shown not to affect the concentration of biogenic amines (Gardini *et al.* (2001)

A growth model developed to predict food spoilage with a mixture of mesophile *Enterobacteriaceae (Escherichia coli, Enterobacter agglomerans, Klebsiella oxytoca, Klebsiella pneumoniae* and *Proteus vulgaris*) and one strain *of Aeromonas hydrophila* was constructed using a quadratic polynomial model (Braun and Sutherland 2005). Growth predicted by this model, however, was markedly slower than the predicted growth for *M. psychrotolerans* (results not shown). Also, predicted growth rates for psychrotolerant bacteria in beef (Giannuzzi *et al*. 1998) were slower from those predicted for *M. psychrotolerans*. None of the available models was able to predict growth of *M. psychrotolerans* or histamine formation in chilled seafood. The development of a new model for growth and histamine formation by *M. psychrotolerans* therefore seems relevant. In fact, further, studies are needed to validate the developed model with naturally contaminated seafood and define the developed models range of applicability with respect to products and storage conditions (Paper 4).

6. Assessment and management of histamine formation in seafood

Control, regulation and information are crucial elements to reduce the frequency of HFP and ensure product safety. EU and USA have established critical limits for concentrations of histamine in seafood and require all seafood dealers and processors to comply with the Hazard Analysis Critical Control Point (HACCP) regulation (FDA/CFSAN 2001c; EC 2005). In this chapter, the current legislation and recommendations within EU and USA are discussed in relation to the new results from the present thesis.

6.1 Control approaches towards formation of histamine in seafood

Temperature is the most important parameter to control histamine formation in fresh fish and seafood products (Chapter 4.2). At the same time, temperature is exposed to the highest risk of fluctuation during storage and distribution. Regulations for both EU and USA aim as preventing high temperature storage and thereby reducing histamine formation in fresh fish and seafood products. According to EU regulation (EC 853/2004) “Fresh fishery products, thawed unprocessed fishery products, and cooked and chilled products from crustaceans and molluscs, must be maintained at a temperature approaching that of melting ice” (EC 2004). With respect to practical seafood inspection this is interpreted as temperatures between 0°C and +2°C (DVFA 2007). The regulation also applies for fishing vessels but the time to reach this temperature is not specified as in the guidance from FDA (Table 6.1). Storage and distribution of lightly preserved seafood within EU are regulated by local authorities and in Denmark products like smoked and gravad products with less than 6% salt and pH above 5 should be stored and distributed at 5°C or below (DVFA 2006). In USA fresh fish and seafood products are to be stored at 4.4°C or below (FDA/CFSAN 2001c). However, if fresh fish are packed in reduced oxygen atmosphere (MAP or vacuum) the temperature limit is reduced to 3.3°C in order to prevent growth of *Clostridium botulinum*. Lightly preserved seafood products, packed in reduced oxygen, can be

stored at 4.4°C if the concentration of salt in the water phase is above 3.5% (FDA/CFSAN 2001a; FDA/CFSAN 2001b).

Table 6.1 Summary of the current FDA guidance for maximum time limits for vessels to chill their fish, to prevent formation of histamine. (Modified from FDA/CFSAN 2001 and http://www.iceyourfish.seagrant.org/haccp.html - accessed June 17th 2007).

	Ice	Ice slurry or refrigerated seawater or brine at 4.4°C	Ice slurry or refrigerated seawater or brine at 10°C
All fish prone to histamine formation < 9 kg	Store in ice within 12 hours of death	Store in slurry or refrigerated seawater within 12 hours of death	Store in slurry or refrigerated seawater within 9 hours of death
All fish prone to histamine formation when water or air temperature is 28.3°C or above	Store in ice 6 hours of death	Store in slurry or refrigerated seawater within 6 hours of death	Store in slurry or refrigerated seawater at 4.4°C or below
Tuna > 9 kg - gutted	Store in ice 6 hours of death	Store in slurry or refrigerated seawater within 6 hours of death	Store in slurry or refrigerated seawater at 4.4°C or below
Tuna > 9 kg - ungutted	Bring internal temperature to 10°C within 6 hours of death, continue chilling until 4.4°C or less	Bring internal temperature to 10°C within 6 hours of death, continue chilling until 4.4°C or less	Use slurry or refrigerated seawater at 4.4°C or below

Within both EU and USA regulations require all seafood dealers and processors that sell fish and seafood products to follow strict monitoring and control procedures to prevent the development of histamine. They have to comply with the HACCP regulations (FDA/CFSAN 2001c; EC 2005). The HACCP principles are based on identification of the hazard followed by pointing out critical control points (CCPs) where specific controls can be applied to prevent, eliminate or minimise risk of food safety hazards. Typical manufacturing CCPs are receiving, cooking, cooling or storing seafood. To ensure that safe products are produced maximum or minimum boundaries, called critical limits are established. For fresh fish and seafood products prone to histamine formation maximum temperatures for storage is an obvious critical limit. In case products surpass the critical limits corrective actions must be implemented. If a cooler fails, for example, a dealer could ice the fish to keep it cold or move it to another cooler. All monitoring activities must be documented on paper, and records are reviewed weekly by a HACCP-trained individual (Huss *et al.* 2004).

6.2 Critical limits for histamine concentrations in seafood

6.2.1 EU

The Commissions regulation (EC) No 2073/2005 of 15. November 2005, on microbiological criteria for foodstuffs (EC 2005) indicates histamine monitoring of fish species of the following families: *Scombridae*, *Clupeidae*, *Engraulidae* and *Coryphaenidae*.

Nine samples must be withdrawn from each batch and concentrations of histamine must comply with the requirements indicated in Table 6.2. Specifically:

- The mean value must not exceed 100 ppm
- Two samples may have a value of more than 100 ppm but less than 200 ppm
- No sample may have a value exceeding 200 ppm.

Table 6.2 Critical limits for histamine in seafood.

Region	Products	Limits[a] m	Limits[a] M	Regulation
EU	Fishery products from fish species associated with a high amount of histidine[b]	100	200	Commission regulation (EC) No 2073/2005 of 15 November on 2005, Chapter 1 Food safety criteria
EU	Fishery products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine[b]	200	400	
USA	Tuna, mahi-mahi and many other species (www.cfsan.fda.gov/~comm/haccp4c1.html - Accessed July 17th, 2007)	50[c]	500[d]	FDA/CFSAN 2001

[a] mg histamine per kg product (ppm). m and M indicate the lower and upper limit respectively.
[b] Particularly fish species of the families: *Scombridae* (bonitos, kingfish, mackerels, seerfish, tunas and wahoo), *Clupeidae* (herrings, shads, sardines and manhadens), *Engraulidae* (anchovies), *Coryphaenidae* (dolphinfishes including mahi-mahi), *Pomatomidae* (bluefishes) and *Scombresosidae* (sauries).
[c] Defect action level
[d] Toxicity level

For fishery products, which have undergone enzymatic maturation treatment in brine other critical limits are applicable (Table 6.2). It is surprising that *Belonidae* (garfish), *Gempylidae* (escolar and oilfish), *Istiophoridae* (marlin and sailfish) and *Xiphiidae* (swordfish) are not mentioned in the EU regulation as these fish species have caused several incidents of HFP (Table 2.7, Table 2.6).

Examinations of histamine must be carried out in accordance with reliable, scientifically recognised methods, such as HPLC. Histamine is the only biogenic amines for which critical limits have been settled.

6.2.2 USA

Because histamine generally is not uniformly distributed in a decomposed fish, a guidance level of 50 ppm has been set in USA. If 50 ppm is found in one section, there is the possibility that other sections may exceed 500 ppm (Table 6.2). The legislation applies to many fish species including escolar, oilfish, and marlins but not garfish, sailfish and swordfish (FDA/CFSAN 2001c). According to the U.S. Food and Drug Administration compliance programs the official fluorometric method from AOAC is used for detection of histamine in seafood (Rogers and Staruszkiewicz 1997).

6.3 Application of the developed models

To demonstrate the potential use of the *M. psychrotolerans* model for management of safety and shelf-life, three examples are provided, where realistic product characteristics and storage conditions are varied. A preliminary software version of the *M. psychrotolerans* model was made in Excel to demonstrate and illustrate its application (see e.g. Figure 6.1).

6.3.1 Temperature

In Figure 6.1 the difference between the recommended storage temperature for fresh vacuum packed fish in EU and USA are illustrated. A critical limit of 100 ppm histamine is used (Table 6.2) to determine the shelf-life. For fresh tuna (pH 5.8) with a low initial concentration of 10 *M. psychrotolerans*/g the predicted time to reach the critical histamine concentration is 3-5 days shorter at 3.3°C compared to at 2.0°C (Figure 6.1). At 0°C the predicted time to formation of 100 ppm histamine in this product is 28 days (results not shown). It is interesting to predict histamine formation under fluctuating temperature storage conditions. The developed model, however, have not yet been validated under such conditions.

Product characteristics and storage conditions	Product 1	Product 2
Morganella psychrotolerans, CFU/g	10	10
Storage period, days	30	30
Temperature, °C	**2.00**	**3.30**
Water phase salt in product, %	0.30	0.30
pH	5.80	5.80
% CO_2 in headspace gas equilibrium	0.0	0.0

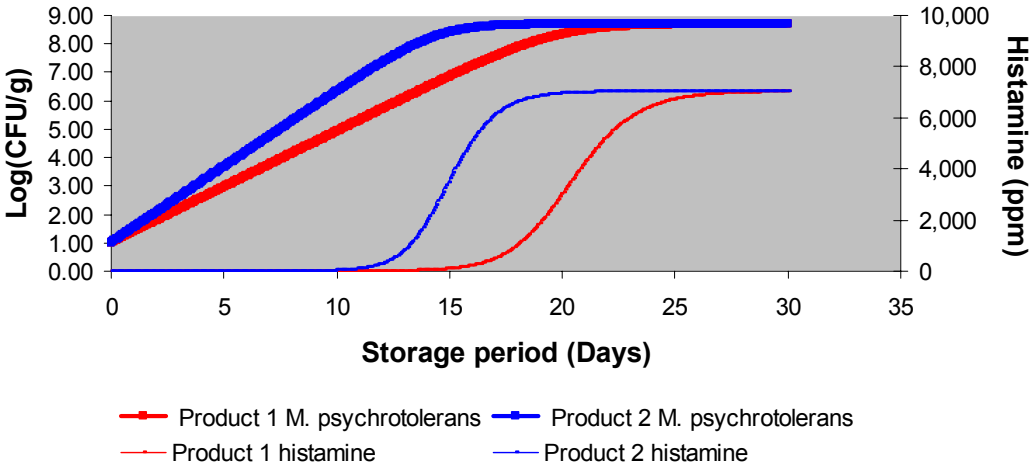


Figure 6.1 Predicted growth (without lag time) and histamine formation in fresh vacuum packed tuna by the *Morganella psychrotolerans* model (Paper 4). Growth (bold lines) and histamine formation (fine lines) was predicted at 2°C (red curves) representing he EU regulation (EC 2004) and at 3.3°C (Blue curves) representing the recommendation by FDA (FDA/CFSAN 2001c).

6.3.2 Initial concentration of *M. psychrotolerans*

HPB have been isolated from skin, gills intestines and muscle tissue of spoiling fish and are considered a natural part of the microflora associated with fish (Chapter 4; Paper 5). The prevalence of *M. psychrotolerans* in fresh fish and in seafood products is however unknown and future surveys of *M. psychrotolerans* are needed. It is not unrealistic to assume that the natural concentration of *M. psychrotolerans* on skin and gills is low and that a slightly higher concentration is present in the intestines. Post harvest contamination of the fresh fish is critical and may occur at several levels; aboard the vessel, at the processing plant, in the distribution system and at the end user (Taylor 1986; Kim *et al*. 2003b). A post harvesting contamination of 10^4 CFU/g and further storage at 5°C in the consumer's home is illustrated in Figure 6.2. The higher initial concentration of *M. psychrotolerans* results in a decreased safe shelf-life with 4 days. It is, however, worth to mention that studies in New Zeeland, Sweden, Ireland, USA, Greece and France have shown that the temperature in home refrigerators fluctuate

and often are 1-2° above the temperature recommended. In some studies 25% of the investigated refrigerators were above 10°C (Sergelidis *et al*. 1997; Laguerre *et al*. 2002; Marklinder *et al*. 2004; Kennedy *et al*. 2005; Gilbert *et al*. 2007; Godwin *et al*. 2007). It has been stated, that the shelf-life of high quality tuna is 11 to 14 days in ice (Kim *et al*. 2004). It is therefore interesting that even with a high initial contamination of *M. psychrotolerans* (10^4 CFU/g), a predicted histamine concentration of 100 ppm is not reached until day 14 (results not shown).

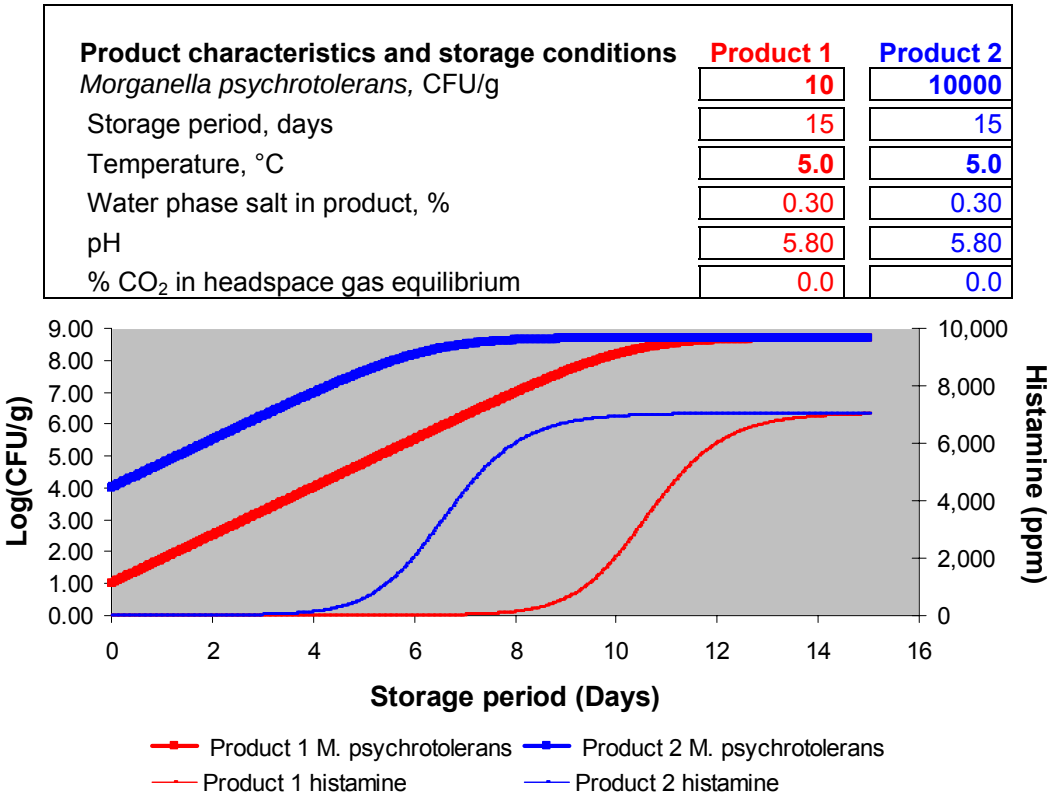


Figure 6.2 Predicted growth (without lag time) and histamine formation in fresh vacuum packed tuna by the *Morganella psychrotolerans* model (Paper 4). Growth (bold lines) and histamine formation (fine lines) were predicted for low (red curves) and high (blue curves) initial concentration of *M. psychrotolerans*.

6.3.3 Product formulation

The *M. psychrotolerans* model can be used to identify combinations of product characteristics and storage conditions that prevent or delay the growth of *M. psychrotolerans* to achieve a desired shelf-life. Figure 6.3 illustrates how adjustment of the water phase salt (WPS) concentration can prolong the safe shelf-life for products like cold-smoked tuna. In this example the 2.2% WPS is derived from an incident of

HFP (Paper 2) and compared to a product with 5% WPS. By adding the right amount of salt a desired shelf-life can be obtained. This clearly demonstrates that the salting process during production of cold-smoked tuna is critical to control the growth and formation of histamine by *M. psychrotolerans*.

Product characteristics and storage conditions	**Product 1**	**Product 2**
Morganella psychrotolerans, CFU/g	**10**	**10**
Storage period (Days)	60	60
Temperature, °C	5.0	5.0
Water phase salt in product, %	**2.2**	**5.0**
pH	5.90	5.90
% CO_2 in headspace gas equilibrium	0.0	0.0

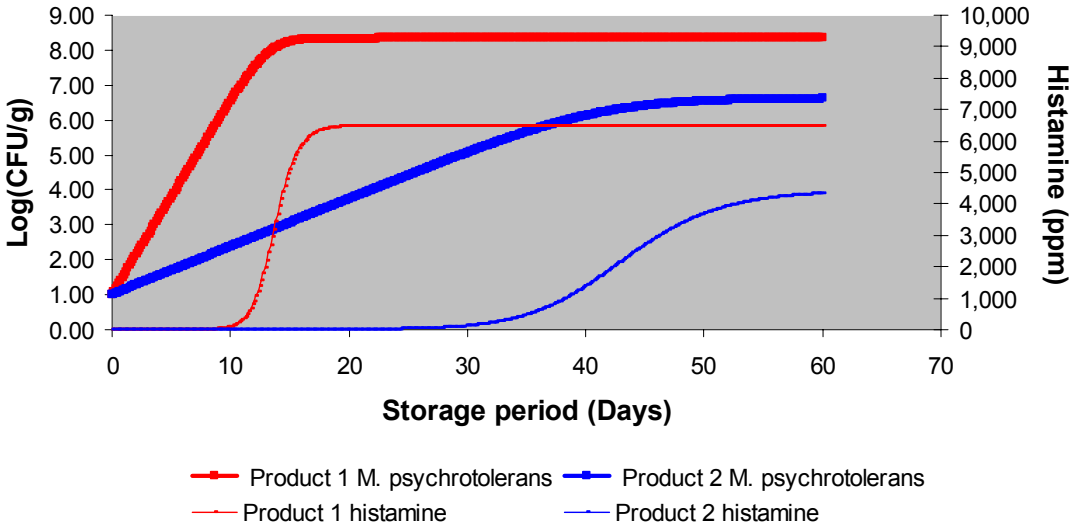


Figure 6.3 Predicted growth (without lag time) and histamine formation in vacuum packed cold-smoked tuna by the *Morganella psychrotolerans* model (Paper 4). Growth (bold lines) and histamine formation (fine lines) were predicted in a product with 2.2% WPS involved HFP (red curves, Paper 2) and in a product with 5% WPS (blue cueves) as suggested in Paper 2.

The suggested WPS of 5% in cold-smoked tuna combined with a shelf-life of no more than 3-4 weeks seems to agree with the prediction (paper 4). However, from the validation of the model it was obvious that further evaluation and possibly improvement of the model are needed. Particular for seafood with added salt, high concentration of CO_2 and products stored at fluctuating temperatures, improvements are needed. Adding the concentration of smoke components as a parameter in the model may improve the accuracy of the model for that type of products. It has been shown that lactic acid bacteria often dominate the spoilage flora of cold smoked seafood products (Sims *et al.*

1992; Paludan-Muller *et al*. 1998; Leroi *et al*. 1998; Hansen and Huss 1998; Jørgensen *et al.* 2000b; Lyhs *et al*. 2002; Olofsson *et al*. 2007). Thus, an extension of the model to include the interaction of lactic acid bacteria and *M. psychrotolerans* may therefore improve the performance of the model.

7. Conclusion and perspectives

Seafood is beneficial for the human health but consumption is also associated with certain risks of diseases, including the risk of histamine fish poisoning (HFP). Even though good statistics on the occurrence are lacking due to underreporting and misdiagnosis, HFP is one of the most frequently observed diseases caused by seafood. Related to the present Ph.D. project, all reported incidents of HFP caused by seafood in Denmark during a three-year period were investigated. The study focused on identification of the histamine-producing bacteria (HPB) present in the seafood and chemical characterisation of the products. In addition, patients answered a questionnaire concerning seafood consumption and symptoms. The typical symptoms observed during the three-year period were flushing, rash, diarrhoea, vomiting and headache. Tuna products (fresh, cold-smoked and canned) caused 69% of the observed incidents and escolar (fresh and smoked) caused 25%. Swordfish caused the last incidents.

It is generally accepted, that concentrations of 500-1,000 ppm histamine cause HFP in the average individual. These findings were supported in the present thesis. Histamine concentration may vary within a batch of fish as well as within a single fish. The findings of 50-100 ppm histamine in one sample may imply that other samples may contain histamine in higher concentrations. Thus, the critical limits (100 ppm in EU and 50 ppm for USA) for histamine in seafood were found appropriate. The evaluation of HFP showed no evidence for establishment of critical limits for biogenic amines other than histamine since proportionality was shown between concentrations of histamine and of other biogenic amines. Furthermore, new investigations with volunteers are required to elucidate the uncertainties concerning the toxic threshold of histamine and the potentiating effects of other biogenic amines.

Identification of the microorganism associated with histamine formation was successful in a total of five Danish incidents (Table 3.1). For the first time, the bacteria responsible for histamine formation in cold smoked tuna were identified. For the remaining incidents either no isolates were obtained at all (no remnants or heat-treated

sample) or no strongly HPB were identified. From 1955 to 2002, the HPB responsible for histamine formation in seafood causing HFP have only been identified in five incidents. Hence, the present Ph.D.-thesis significantly increases the knowledge about the specific HPB that actually causes HFP. It was shown that psychrotolerant HPB might be as important as mesophile HPB with respect to histamine formation in seafood causing HFP in Denmark. A strongly histamine-producing and psychrotolerant variant of *M. morganii* was isolated in two incidents of HFP. The organism was identified during the present Ph.D. study using a polyphasic approach including multi locus sequence and recognised as a new species of *Morganella.* This new species was named *Morganella psychrotolerans*. Fragments of seven protein-encoding housekeeping genes all showed less than 90.9% similarity between the two groups of *M. morganii* and *M. psychrotolerans*. *M. psychrotolerans* differ from *M. morganii* with respect to growth at 0°C and 37°C or in 8.5% NaCl (w/v) and fermentation of D-galactose. Gene-sequences-analysis revealed the possibility for subdivision of *M. psychrotolerans* just as with *M. morganii*. However, further studies with new strains of *M. psychrotolerans* are needed before such a division can be applied. Specific detection of *M. psychrotolerans* is needed to determine its prevalence in seafood, marine environments, processing facilities and to determine the natural habitat. A specific detection method would also (i) assist the further investigation of the strongly HPB causing incidents of HFP, which are needed to support the findings of this thesis and (ii) improve the usefulness of the developed models for growth and formation of histamine by *M. psychrotolerans*.

A predictive model for the growth and histamine formation by *M. psychrotolerans* was developed. As primary model the expanded logistic model with a dampening factor (*m*) fixed to 0.7 was used. A square-root type model including cardinal parameters modelled the growth rate of *M. psychrotolerans*. This model included the effect of storage conditions (temperature (0-25°C) and CO_2 (0-100%)) and product characteristic (pH (5.4-6.5) and NaCl (0-5%)). A constrained polynomial model successfully modelled the effect of the environmental parameters (CO_2 and NaCl) on the maximum population density (log N_{max}). Histamine formation was modelled by relating it to growth of *M. psychrotolerans* with a constant yield factor ($Y_{His/CFU}$). Log $Y_{His/CFU}$ was proportional to log N_{max} under different environmental conditions. This is the first model for histamine formation, which considers the effect of four parameters. The model predicted the average time to formation of 500 ppm histamine for fresh fish and liquid cultures with a deviation of ± 10%. On the other hand, further validation studies

and more storage trials of lightly preserved seafood products like cold-smoked tuna are needed before the model can be applied to that group of products. In addition evaluation of the model used under dynamic conditions are needed to further improve the model. The model could be further developed by adding terms for the effect of smoke components. For quantitative risk assessment of histamine formation in seafood, development of equivalent models of histamine formation for other strongly HPB especially, *P. phosphoreum* and *M. morganii* are needed. Finally, uncertainty and variability of predictions can be studied to improve the applicability of the models for assessment and management of risks.

Storage trials showed that psychrotolerant HPB formed histamine in toxic concentration in vacuum-packed tuna stored at ~2°C within 12-14 days. However, it was, also shown that modified atmosphere packaging with O_2 and CO_2 was an efficient way to reduce the growth and the concomitant formation of histamine of both *M. psychrotolerans* and *P. phosphoreum* in fresh tuna and could improve the safety of fresh tuna and other fishes. More studies are needed to determine if this preserving technique is as efficient at temperatures higher than 2°C.

The present Ph.D. thesis has provided crucial information on psychrotolerant HPB and their importance for HFP. A new psychrotolerant and strongly histamine-producing species; *M. psychrotolerans* was isolated from seafood causing HFP. Furthermore, it was *demonstrated* that psychrotolerant HPB might be as important as mesophilic HPB with respect to HFP. It was suggested that a modified atmosphere with O_2 and CO_2 can be used instead of vacuum packaging since growth-inhibition of *M. psychrotolerans* and *P. phosphoreum* was observed. In cold smoked tuna more than 5% WPS are needed in combination with at shelf-life of no more than 3-4 weeks to prevent toxic concentrations of histamine in the product. Finally, a predictive model for the growth and histamine formation by *M. psychrotolerans* was developed. This model is the first step towards a quantitative exposure assessment and may work as a template for similar models for other HPB. The model will be included in the software SSSP, from where, it can be used as an important decision tools by the seafood industry as well as regulatory authorities.

8. References

Ababouch, L., Afilal, M. E., Benandeljelil, H. and Busta, F. F. 1991a Quantitative changes in bacteria, amino-acids and biogenic amines in sardine (*Sardina pilchardus*) stored at ambient temperature (25-28°C) and in ice. *International Journal of Food Science and Technology* 26, 297-306.

Ababouch, L., Afilal, M. E., Rhafiri, S. and Busta, F. F. 1991b Identification of histamine-producing bacteria isolated from sardine (*Sardina pilchardus*) stored in ice and at ambient temperature (25°C). *Food Microbiology* 8, 127-136.

Ababouch, L., Souibri, L., Rhaliby, K., Ouahdi, O., Battal, M. and Busta, F. F. 1996 Quality changes in sardines (*Sardina pilchardus*) stored in ice and at ambient temperature. *Food Microbiology* **13**, 123-132.

Aiiso, K., Toyoura, H. and Iida, H. 1958 Distribution and activity of histidine decarboxylase in *Morganella*. *Japanese Journal of Microbiology* 2, 143-147.

Anonymous 2004 Advice on Fish Consumption: Benefits & Risks, The Stationary Office, Food Standards Agency and the Department of Health, London, UK.

AOAC 2005 Histamine in seafood, fluorometric method, 977.13. *Official Methods of the analysis of AOAC International* 18.

Arnold, S. H. and Brown, W. D. 1978 Histamine (?) Toxicity from fish products. *Advances in Food Research* 24, 113-154.

Arnold, S. H., Price, R. J. and Brown, W. D. 1980 Histamine formation by bacteria isolated from skipjack tuna (*Katsuwonus pelamis*). *Bulletin of the Japanese Society of Scientific Fisheries* 46, 991-995.

Askar, A. and Treptow, H. 1986 Biogene Amine in Lebensmitteln: Vorkommen, Bedeutung und Bestimmung. Eugen Ulmer Verlag, Stuttgart, Germany.

Aytac, S. A., Ozbas, Z. Y. and Vural, H. 2000 Effects of irradiation, antimicrobial agents and modified-atmosphere packaging on histamine production by *Morganella morganii* in mackerel fillets. *Archiv für Lebensmittelhygiene* 51, 28-30.

Bailey, J. E. and Ollis, D. F. 1986 Biochemical Engineering Fundamentals. 2 edn, McGraw-Hill Book Company, New York.

Barancin, C. E., Smoot, J. C., Findlay, R. H. and Actis, L. A. 1998 Plasmid-mediated histamine biosynthesis in the bacterial fish pathogen *Vibrio anguillarum*. *Plasmid* 39, 235-244.

Baranowski, J. D. 1985 Low-temperature production of urocanic acid by spoilage bacteria isolated from mahi-mahi (*Corphaena hippurus*). *Applied and Environmental Microbiology* 50, 546-547.

Baranyi, J., Ross, T., McMeekin, T. A. and Roberts, T. A. 1996 Effects of parameterization on the performance of empirical models used in "predictive microbiology". *Food Microbiology* 13, 83-91.

Baranyi, J. and Roberts, T. A. 1995 Mathematics of predictive food microbiology. *International Journal of Food Microbiology* 26, 199-218.

Baranyi, J. and Roberts, T. A. 1994 A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology* 23, 277-294.

Bardocz, S. 1995 Polyamines in food and their consequences for food quality and human health. *Trends in Food Science & Technology* 6, 341-346.

Barrow, G. I. and Feltham, R. K. A. 1999 Cowan and Steel's Manual for the Identification of Medical Bacteria. 3 edn, Cambridge University Press, New York.

Bartholomew, B. A., Berry, P. R., Rodhouse, J. C. and Gilbert, R. J. 1987 Scombroid fish poisoning in Britain: Features of over 250 suspected incidents from 1976 to 1986. *Epidemiology and infection* 99, 775-782.

Becker, K., Southwick, K., Reardon, J., Berg, R. and MacCormack, J. N. 2001 Histamine poisoning associated with eating tuna burgers. *Journal of the American Medical Association* 285, 1327-1330.

Bédry, R., Gabinski, C. and Paty, M.-C. 2000 Diagnosis of scrombroid poisoning by measurement of plasma histamine. *New England Journal of Medicine* 342, 520-521.

Behling, A. R. and Taylor, S. L. 1982 Bacterial histamine production as a function of temperature and time of incubation. *Journal of Food Science* 47, 1311-1317.

Ben Gigirey, B., de Sousa, J. M. V. B., Villa, T. G. and Barros-Velazquez, J. 1999 Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). *Journal of Food Protection* 62, 933-939.

Ben Gigirey, B., Vieites Baptista de Sousa, J. M., Villa, T. G. and Barros-Velazquez, J. 2000 Characterization of biogenic amine-producing *Stenotrophomonas maltophilia* strains isolated from white muscle of fresh and frozen albacore tuna. *International Journal of Food Microbiology* 57, 19-31.

Bermejo, A., Mondaca, M. A., Roeckel, M. and Marti, M. C. 2004 Bacterial formation of histamine in jack mackerel (*Trachurus symmetricus*). *Journal of Food Processing and Preservation* 28, 201-222.

Bermejo, A., Mondaca, M. A., Roeckel, M. and Marti, M. C. 2002 Growth and characterization of the histamine-forming bacteria of jack mackerel (*Trachurus symmetricus*). *Journal of Food Processing and Preservation* 26, 401-414.

Blakesley, M. L. 1983 Scrombroid poisoning: Promt resolution of symptoms with cimetidine. *Annals of Emergency Medicine* 12, 104-106.

Boutin, J.-P., Puyhardy, J.-M., Chianea D., Andreu, P., Paez, S., Fize, L., Vauthier, J.-M., Chapalain, J.-C., Grippari, J.-L., Corbe, H. and Bietrix, P. 1998 Histamine food poisoning. *Santé publique* 10, 29-37.

Braun, P. and Sutherland, J. P. 2005 Predictive modelling of growth and measurement of enzymatic synthesis and activity by a cocktail of selected Enterobacteriaceae and *Aeromonas hydrophila*. *International Journal of Food Microbiology* 105, 257-266.

Bremer, P. J., Fletcher, G. C. and Osborne, C. M. 2003 Scombrotoxin in Seafood, New Zealand Institute for Crop & Food Research Limited.

Buffoni, F. 1966 Histaminase and related amine oxidases. *Pharmacological Reviews* 18, 1163-1199.

Caston, J. C., Eaton, C. L., Gheorghiu, B. P. and Ware, L. W. 2002 Tyramine induced hypertensive episodes and panic attacks in hereditary deficient monoamine oxidase patients: Case reports. *The Journal of the South Carolina Medical Association* 98, 184-189.

Clifford, M. N., Walker, R., Ijomah, P., Wright, J., Murray, C. K. and Hardy, R. 1991 Is there a role for amines other than histamines in the etiology of scombrotoxicosis? *Food Additives and Contaminants* 8, 641-652.

Clifford, M. N., Walker, R., Wright, J., Hardy, R. and Murray, C. K. 1989 Studies with volunteers on the role of histamine in suspected scombrotoxicosis. *Journal of the Science of Food and Agriculture* 47, 365-375.

Corlett, D. A. Jr., Jeffrey, M. B. and Niven, C. F. 1978 Identity of histamine-forming bacteria from scombroid fish. *Abstracts of the Annual Meeting of the American Society for Microbiology* 78, 192.

Coton, E., Rollan, G. C. and Lonvaud-Funel, A. 1998 Histidine carboxylase of *Leuconostoc oenos* 9204: Purification, kinetic properties, cloning and nucleotide sequence of the hdc gene. *Journal of Applied Microbiology* 84, 143-151.

CSPI 2006 "Outbreak Alert - Closing the Gaps in Our Federal Food-Safety Net" Center for Science in the Public Interest (CSPI), Washington DC, USA.

Dalgaard, P. 2006 "Microbiology of marine muscle foods" in *Handbook of Food Science, Technology, and Engineering*, Y. H. Hui, ed., CRC Press, Boca Raton, FL, USA, pp. 53-1-53-20.

Dalgaard, P. 1995a Modelling of microbial activity and prediction of shelf life for packed fresh fish. *International Journal of Food Microbiology* 26, 305-317.

Dalgaard, P. 1993 Evaluation and Prediction of Microbial Fish Spoilage (Ph.D-thesis). Technological Laboratory, Danish Ministry of Fisheries, Lyngby, Denmark.

Dalgaard, P. 1995b Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology* 26, 319-333.

Dalgaard, P. 2002 "Modelling and predicting the shelf-life of seafood" in *Safety and Quality Issues in Fish Processing*, H. A. Bremner, ed., Woodhead Publishing Ltd., Cambridge, England, pp. 191-219.

Dalgaard, P., Madsen, H. L., Samieian, N. and Emborg, J. 2006 Biogenic amines formation and microbial spoilage in chilled garfish (*Belone belone belone*) - effect of modified atmosphere packaging and previous frozen storage. *Journal of Applied Microbiology* 101, 80-95.

Dalgaard, P., Mejlholm, O. and Huss, H. H. 1997 Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. *International Journal of Food Microbiology* 38, 169-179.

Dalgaard, P., Murillo, E. I. and Jørgensen, L. V. 2004 "Modelling the effect of temperature on shelf-life and on the interaction between the spoilage microflora and *Listeria monocytogenes* in cold-smoked salmon" in *Seafood Quality and Safety - Advances in the New Millennium*, F. Shahidi & B. K. Simpson, eds., ScienceTech Publishing Company, St. John's, NL, Canada, pp. 281-302.

Dawood, A. A., Karkalas, J., Roy, R. N. and Williams, C. S. 1988 The occurrence of non-volatile amines in chilled-stored rainbow-trout (*Salmo irideus*). *Food Chemistry* 27, 33-45.

de las Rivas, B., Marcobal, A., Carrascosa, A. V. and Munoz, R. 2006a PCR detection of foodborne bacteria producing the biogenic amines histamine, tyramine, putrescine, and cadaverine. *Journal of Food Protection* 69, 2509-2514.

de las Rivas, B., Marcobal, A. and Munoz, R. 2006b Gene organization of the ornithine decarboxylase-encoding region in *Morganella morganii*. *Journal of Applied Microbiology* 102, 1551-1560.

DeWaal, C. S., Hicks, G., Barlow, K., Alderton, L. and Vegosen, L. 2006 Foods associated with food-borne illness outbreaks from 1990 through 2003. *Journal of Food Protection* 26, 466-473.

Du, W. X., Huang, T. S., Kim, J., Marshall, M. R. and Wei, C. I. 2001 Chemical, microbiological, and AromaScan evaluation of mahi-mahi fillets under various storage conditions. *Journal of Agricultural and Food Chemistry* 49, 527-534.

DVFA 1997 http://www.foedevarestyrelsen.dk/fdir/publications/1998300/Rapport1.asp (in Danish) Accessed 11.07.2007.

DVFA 1999 http://www.foedevarestyrelsen.dk/FDir/Publications/1999006/Rapport.htm (in Danish) Accessed 11.07.2007.

DVFA 2000 Reported outbreaks of foodborne diseases 1999 (in Danish), Danish Veterinary and Food Administration, FødevareRapoprt 2000:27.

DVFA 2001a Reported outbreaks of foodborne diseases 2000 (in Danish), Danish Veterinary and Food Administration, FødevareRapport 2001:13.

DVFA 2001b Reported outbreaks of foodborne diseases 2001 (in Danish), Danish Veterinary and Food Administration, FødevareRapport 200212.

DVFA 2003 Generel view at fish and seafood products (in Danish), Danish Veterinary and Food Administration, Shultz, Denmark, FødevareRapport 2003:17.

DVFA 2004 Reported outbreaks of foodborne diseases 2002 (in Danish), Danish veterianary and Food Administration, FødevareRapport 2004:01.

DVFA 2005 Reported outbreaks of foodborne diseases 2003 (in Danish), Danish Veterinary and Food Administration, FødevareRaport 2005:05.

DVFA 2006 Announcement on Food Hygiene BEK nr 773 of 03/07/2006 (in Danish), Danish Veterinary and Food Administration.

DVFA 2007 Guidance on Food Hygiene VEJ nr 9355 of 01/06/2007 (in Danish), Danish Veterinary and Food Administration.

EC 2004 Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Union* 226, 22-82.

EC 2005 Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union* 338, 1-25.

EFSA 2005 EFSA provides advice on the safety and nutritional contribution of wild and farmed fish, European Food Safety Authority, Parma, Italy.

Eitenmiller, R. R., Wallis, J. W., Orr, J. H. and Phillips, R. D. 1981 Production of histidine decarboxylase and histamine by *Proteus morganii*. *Journal of Food Protection* 44, 815-820.

El-Marrakchi, A., Bennour, M., Bouchriti, N., Hamama, A. and Tagafait, H. 1990 Sensory, chemical and microbiological assessments of Moroccan sardines (*Sardina pilchardus*) stored in ice. *Journal of Food Protection* **53**, 600-605.

Emborg, J. and Dalgaard, P. 2007 Seafood biogenic amine database (in preparation) SEAFOODplus, Deliverable D3. Danish Institute for Fisheries Research.

Emborg, J., Dalgaard, P., Kjølby, A., Sørensen, N. D., Ballin, N. Z. and Larsen, I. K. 2006 Biogenic amine concentrations, microflora and product characteristics of seafoods implicated in incidents of histamine fish poisoning (HFP), SEAFOODplus, November 2006, Deliverable D4. Danish Institute for Fisheries Research. pp 22.

Emborg, J., Laursen, B. G., Rathjen, T. and Dalgaard, P. 2002 Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2°C. *Journal of Applied Microbiology* 92, 790-799.

Espe, M., Lied, E. and Torrissen, K. R. 1993 Changes in plasma and muscle free amino-acids in Atlantic salmon (*Salmo salar*) during absorption of diets containing different amounts of hydrolyzed cod muscle protein. *Comparative Biochemistry and Physiology A-Physiology* 105, 555-562.

Etkind, P., Wilson, M. E., Gallagher, K. and Cournoyer, J. 1987 Bluefish-associated scombroid poisoning - an example of the expanding spectrum of food poisoning from seafood. *Journal of the American Medical Association* 258, 3409-3410.

FDA/CFSAN 2001a "*Clostridium botulinum* toxin formation (a biological hazard)" in *Fish and Fishery Products Hazards and Controls Guidance*, 3 edn, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC, pp. 167-189.

FDA/CFSAN 2001b "Pathogen growth & toxin formation (other that *Clostridium botulinum*) as a result of time/temperature abuse" in *Fish and Fishery Products Hazards and Controls Guidance*, 3 edn, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC, pp. 167-189.

FDA/CFSAN 2001c "Scombrotoxin (histamine) formation" in *Fish and Fishery Products Hazards and Controls Guidance*, 3 edn, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC, pp. 83-102.

Feldman, K. A., Werner, S. B., Cronan, S., Hernandez, M., Horvath, A. R., Lea, C. S., Au, A. M. and Vugia, D. J. 2005 A large outbreak of scombroid fish poisoning associated with eating escolar fish (*Lepidocybium flavobrunneum*). *Epidemiology and infection* 133, 29-33.

Ferencik, M. 1970 Formation of histamine during bacterial decarboxylation of histidine in flesh of some marine fishes. *Journal of Hygiene Epidemiology Microbiology and Immunology* 14, 52-60.

Fernández-Salguero, J. and Mackie, I. M. 1979 Histidine metabolism in mackerel (*Scomber-scombrus*) - studies on histidine-decarboxylase activity and histamine formation during storage of flesh and liver under sterile and non-sterile conditions. *Journal of Food Technology* 14, 131-139.

Fletcher, G. C., Summers, G., Winchester, R. V. and Wong, R. J. 1995 Histamine and histidine in New Zealand marine fish and shellfish species, particularly kahawai (*Arripis trutta*). *Journal of Aquatic Food Product Technology* **4**, 53-74.

Francois, K., Devlieghere, F., Standaert, A. R., Geeraerd, A. H., Cools, I., Van Impe, J. F. and Debevere, J. 2005 Environmental factors influencing the relationship between optical density and cell count for *Listeria monocytogenes*. *Journal of Applied Microbiology* 99, 1503-1515.

Frank, H. A., Baranowski, J. D., Chongsiriwatana, M., Brust, P. A. and Premaratne, R. J. 1985 Identification and decarboxylase activities of bacteria isolated from decomposed mahi-mahi (*Coryphaena hippurus*) after incubation at 0°C and 32°C. *International Journal of Food Microbiology* 2, 331-340.

Frank, H. A. and Yoshinaga, D. H. 1987 Table for estimating histamine formation in skipjack tuna, *Katsuwonus pelamis*, at low nonfreezing temperature. *Marine Fisheries Review* **49**, 67-70.

Frank, H. A., Yoshinaga, D. H. and Nip, W. 1981 Histamine formation and honeycombing during decomposition of skipjack tuna, *Katsuwonus pelamis*, at elevated temperatures. *Marine Fisheries Review* 43, 9-14.

Frank, H. A., Yoshinaga, D. H. and Wu, I. P. 1983 Nomograph for estimating histamine formation in skipjack tuna at elevated temperatures. *Marine Fisheries Review* 45, 40-44.

Fujii, T., Hiraishi, A., Kobayashi, T., Yoguchi, R. and Okuzumi, M. 1997 Identification of the psychrophilic histamine-producing marine bacteria previously referred to as the N-group bacteria. *Fisheries Science* 63, 807-810.

Gardini, F., Martuscelli, M., Caruso, M. C., Galgano, F., Crudele, M. A., Favati, F., Guerzoni, M. E. and Suzzi, G. 2001 Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic

activity and biogenic amine production of *Enterococcus faecalis*. *International Journal of Food Microbiology* 64, 105-117.

Geeraerd, A. H., Valdramidis, V. P., Devlieghere, F., Bernaert, H., Debevere, J. and Van Impe, J. F. 2004 Development of a novel approach for secondary modelling in predictive microbiology: Incorporation of microbiological knowledge in black box polynomial modelling. *International Journal of Food Microbiology* 91, 229-244.

Geiger, E. 1944 Histamine content of unprocessed and canned fish. A tentative method for quantitative determination of spoilage. *Food Research* 9, 293-297.

Geng, T., Kim, K. P., Gomez, R., Sherman, D. M., Bashir, R., Ladisch, M. R. and Bhunia, A. K. 2003 Expression of cellular antigens of *Listeria monocytogenes* that react with monoclonal antibodies C11E9 and EM-7G1 under acid-, salt- or temperature-induced stress environments. *Journal of Applied Microbiology* 95, 762-772.

Gessner, B. D., Hokama, Y. and Isto, S. 1996 Scombrotoxicosis-like illness following the ingestion of smoked salmon that demonstrated low histamine levels and high toxicity on mouse bioassay. *Clinical Infectious Diseases* 23, 1316-1318.

Giannuzzi, L., Pinotti, A. and Zaritzky, N. 1998 Mathematical modelling of microbial growth in packaged refrigerated beef stored at different temperatures. *International Journal of Food Microbiology* 39, 101-110.

Gibson, A. M., Bratchell, N. and Roberts, T. A. 1988 Predicting microbial-growth - growth-responses of salmonellae in a laboratory medium as affected by pH, sodium-chloride and storage-temperature. *International Journal of Food Microbiology* 6, 155-178.

Gilbert, R. J., Hobbs, G., Murray, C. K., Cruickshank, J. G. and Young, S. E. J. 1980 Scombrotoxic fish poisoning - Features of the first 50 incidents to be reported in Britain (1976-9). *British Medical Journal* 281, 71-72.

Gilbert, S. E., Whyte, R., Bayne, G., Lake, R. J. and van der Logt, P. 2007 Survey of internal temperatures of New Zealand domestic refrigerators. *British Food Journal* 109, 323-329.

Gillespie, I. A., Adak, G. K., O'Brien, S. J., Brett, M. and Bolton, F. J. 2001 General outbreaks of infectious intestinal disease associated with fish and shellfish, England and Wales, 1992-1999. *Communicable Disease and Public Health* 4, 117-123.

Gingerich, T. M., Lorca, T., Flick, G. J., Pierson, M. D. and McNair, H. M. 1999 Biogenic amine survey and organoleptic changes in fresh, stored, and temperature-abused bluefish (*Pomatomus saltatrix*). *Journal of Food Protection* 62, 1033-1037.

Gingerich, T. M., Lorca, T., Flick, G. J. Jr., McNair, H. M. and Pierson, M. D. 2001 Isolation of histamine-producing bacteria from fish-processing facilities and fishing vessels. *Journal of Aquatic Food Product Technology* 10, 61-66.

Glória, M. B. A. 2006 "Bioactive amines" in *Handbook of Food Science, Technology and Engineering*, 1 edn, vol. 1 Y. H. Hui, ed., CRC Press, Boca Rato, FL, USA, p. 13.1-13.37.

Godwin, S. L., Chen, F.-C., Chambers E. and Coppings, R. 2007 A comprenhensive evaluation of tempeartures within home refrigerators. *Food Protecting Trends* 27, 168-173.

Guirard, B. M. and Snell, E. E. 1987 Purification and properties of pyridoxal-5'-phosphate-dependent histidine decarboxylases from *Klebsiella planticola* and *Enterobacter aerogenes*. *Journal of Bacteriology* 169, 3963-3968.

Guizani, N., Al-Busaidy, M. A., Al-Belushi, I. M., Mothershaw, A. and Rahman, M. S. 2005 The effect of storage temperature on histamine production and the freshness of yellowfin tuna (*Thunnus albacares*). *Food Research International* 38, 215-222.

Hackert, M. L., Meador, W. E., Oliver, R. M., Salmon, J. B., Recsei, P. A. and Snell, E. E. 1981 Crystallization and subunit structure of histidine decarboxylase from *Lactobacillus* 30a. *Journal of Biological Chemistry* 256, 687-690.

Halász, A., Baráth, Á., Simon-Sakardi, L. and Holzapfel, W. H. 1994 Biogenic amines and their production by microorganisms in food. *Trends in Food Science & Technology* 5, 42-49.

Hansen, L. T. and Huss, H. H. 1998 Comparison of the microflora isolated from spoiled cold-smoked salmon from three smokehouses. *Food Research International* 31, 703-711.

Hauser, M. J. and Baier, H. 1982 Interactions of isoniazid with foods. *Drug Intelligence & Clinical Pharmacy* 16, 617-618.

Havelka, B. 1967 The role of bacteria from Hafnia genus in the origination of histamine in tunny meat. *Ceskoslovenska hygiena* 12, 343-352.

Hazeleger, W. C., Dalvoorde, M. and Beumer, R. R. 2006 Fluorescence microscopy of NaCl-stressed, elongated *Salmonella* and *Listeria* cells reveals the presence of septa in filaments. *International Journal of Food Microbiology* 112, 288-290.

Hernández-Herrero, M. M., Roig-Sagués, A. X., Rodríguez-Jerez, J. J. and Mora-Ventura, M. T. 1999 Halotolerant and halophilic histamine-forming bacteria isolated during the ripening of salted anchovies (*Engraulis encrasicholus*). *Journal of Food Protection* 62, 509-514.

Hesterberg, R., Sattler, J., Lorenz, W., Stahlknecht, C.-D., Barth, H., Crombach, M. and Weber, D. 1984 Histamine content, diamine oxidase activity and histamine methyltransferase activity in human tissues: fact and fiction? *Agents and Actions* 14, 325-334.

Hibiki, S. and Simidu, W. 1959 Studies on putrefaction of aquatic products. 27. Inhibition of histamine formation in spoiling of cooked fish and histidine content in various fishes. *Bulletin of the Japanese Society of Scientific Fisheries* 24, 916-919.

Hug, D. H. and Hunter, J. K. 1974 Effect of temperature on histidine ammonia-lyase from a psychrophile, *Pseudomonas putida*. *Journal of Bacteriology* 119, 92-97.

Hughes, C., Gillespie, I. A. and O'Brien, S. J. 2007 Foodborne transmission of infectious intestinal disease in England and Wales, 1992-2003. *Food Control* 18, 766-772.

Hui, J. Y. and Taylor, S. L. 1985 Inhibition of invivo histamine-metabolism in rats by foodborne and pharmacologic inhibitors of diamine oxidase, histamine n-methyltransferase, and monoamine-oxidase. *Toxicology and Applied Pharmacology* 81, 241-249.

Huis in´t Veld, J. H. J., Hose, H., Schaafsma, G. J., Silla, H. and Smith, J. E. 1990 "Health aspects of food biotechnology" in *Processing and Quality of Foods*, vol. 2, Food Biotechnology: Avenues to Healthy and Nutritious Products P. Zeuten et al., eds., Elsevier Applied Science, London and New York, p. 2.73-2.97.

Huss, H. H. 1995 Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper No. 348. FAO, Rome.

Huss, H. H., Ababouch, L. and Gram, L. 2004 Assessment and Management of Seafood Safety and Quality. FAO Fisheries Technical Paper No. 444. FAO, Rome.

Huynh, Q. K. and Snell, E. E. 1985 Pyruvoyl-dependent histidine decarboxylases. Preparation and amino acid sequences of the beta chains of histidine decarboxylase from *Clostridium perfringens* and *Lactobacillus buchneri*. *Journal of Biological Chemistry* 260, 2798-2803.

Hwang, D. F., Chang, D. S., Shiau, C. Y. and Cheng, C. C. 1995 Biogenic-amines in the flesh of sailfish (*Istiophorus-platypferus*) responsible for scombroid poisoning. *Journal of Food Science* 60, 926-928.

Hwang, D. F., Chang, S. H., Shiua, C. Y. and Tuu-jyi, C. 1997 High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. *Journal of Chromatography B: Biomedical Sciences and Applications* 693, 23-30.

Ijomah, P., Clifford, M. N., Walker, R., Wright, J., Hardy, R. and Murray, C. K. 1991 The importance of endogenous histamine relative to dietary histamine in the etiology of scombrotoxicosis. *Food Additives and Contaminants* 8, 531-542.

Janda, J. M. and Abbott, S. L. 2005 "*Genus* XXI. Morganella *Fulton* 1943, 81^{AL}" in *Bergye's manual of systematic bacteriology*, 2. edn, vol. 2 part B G. M. Garrity et al., eds., pp. 707-709.

Jørgensen, L. V., Dalgaard, P. and Huss, H. H. 2000a Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *Journal of Agricultural and Food Chemistry* 48, 2448-2453.

Jørgensen, L. V., Huss, H. H. and Dalgaard, P. 2000b The effect of biogenic amine production by single bacterial cultures and metabiosis in cold-smoked salmon. *Journal of Applied Microbiology* 89, 920-934.

Kaliner, M., Shelhamer, J. H. and Ottesen, E. A. 1982 Effects of infused histamine - correlation of plasma histamine levels and symptoms. *Journal of Allergy and Clinical Immunology* 69, 283-289.

Kamath, A. V., Vaaler, G. L. and Snell, E. E. 1991 Pyridoxal phosphate-dependent histidine decarboxylases - cloning, sequencing, and expression of genes from *Klebsiella planticola* and *Enterobacter aerogenes* and properties of the overexpressed enzymes. *Journal of Biological Chemistry* 266, 9432-9437.

Kan, K., Ushiyama, H., Shindo, T., Uehara, S. and Yasuda, K. 2000 Outbreak of histamine poisoning due to ingestion of fish, "Abura-sokomutsu" (*Lepidocybium flavobrunneum*). *Journal of the Food Hygienic Society of Japan* 41, 116-121.

Kanki, M., Yoda, T., Ishibashi, M. and Tsukamoto, T. 2004 *Photobacterium phosphoreum* caused a histamine fish poisoning incident. *International Journal of Food Microbiology* 92, 79-87.

Kanki, M., Yoda, T., Tsukamoto, T. and Shibata, T. 2002 *Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. *Applied and Environmental Microbiology* 68, 3462-3466.

Kanki, M., Yoda, T., Tsukamoto, T. and Baba, E. 2007 Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. *Applied and Environmental Microbiology* 73, 1467-1473.

Kawabata, T., Ishizaka, K. and Miura, T. 1955b Studies on the food poisoning associated with putrefaction of marine products - I. Outbreaks of allergy-like food poisoning caused by "Samma sakuraboshi" (dried seasoned saury) and canned seasoned mackerel. *Bulletin of the Japanese Society of Scientific Fisheries* 21, 335-349.

Kawabata, T., Ishizaka, K. and Miura, T. 1955a Studies on the allergy-like food poisoning associated with putrefaction of marine products.I.Episodes of allergy-like food poisoning caused by "Samma sakuraboshi" (dried seasoned saury) and other kinds of marine products. *Japanes Jourmal of Medical Science and Biology* 487-501.

Kennedy, J., Jackson, V., Blair, I. S., McDowell, D. A., Cowan, C. and Bolton, D. J. 2005 Food safety knowledge of consumers and the microbiological and temperature status of their refrigerators. *Journal of Food Protection* 68, 1421-1430.

Kerr, M., Lawicki, P., Aguirre, S. and Rayner, C. 2002 Effect of storage conditions on histamine formation in fresh and canned tuna, Public Health Division, Victorian Government Department of Human Services, www.foodsafety.vic.gov.au.

Kim, I. S. and Bjeldanes, L. F. 1979 Amine content of toxic and wholesome canned tuna fish. *Journal of Food Science* 44, 922-923.

Kim, S. H., An, H. J., Field, K. G., Wei, C. I., Velazquez, J. B., Ben Gigirey, B., Morrissey, M. T., Price, R. J. and Pitta, T. P. 2003a Detection of *Morganella morganii,* a prolific histamine former, by the polymerase chain reaction assay with 16S rDNA-targeted primers. *Journal of Food Protection* 66, 1385-1392.

Kim, S. H., An, H. J. and Price, R. J. 1999 Histamine formation and bacterial spoilage of albacore harvested off the U.S. northwest coast. *Journal of Food Science* 64, 340-343.

Kim, S. H., Barros-Velazquez, J., Ben Gigirey, B., Eun, J. B., Jun, S. H., Wei, C. I. and An, H. J. 2003b Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. *Food Science and Biotechnology* 12, 451-460.

Kim, S. H., Ben Gigirey, B., Barros-Velazquez, J., Price, R. J. and An, H. J. 2000 Histamine and biogenic amine production by *Morganella morganii* isolated from temperature-abused albacore. *Journal of Food Protection* 63, 244-251.

Kim, S. H., Field, K. G., Chang, D. S., Wei, C. I. and An, H. J. 2001a Identification of bacteria crucial to histamine accumulation in Pacific mackerel during storage. *Journal of Food Protection* 64, 1556-1564.

Kim, S. H., Field, K. G., Morrissey, M. T., Price, R. J., Wei, C. I. and An, H. J. 2001b Source and identification of histamine-producing bacteria from fresh and temperature-abused albacore. *Journal of Food Protection* 64, 1035-1044.

Kim, S. H., Morrissey, M. T., Field, K. G., Wei, C. I. and An, H. J. 2001c Occurrence of histamine-forming bacteria in albacore and histamine accumulation in muscle at ambient temperature. *Journal of Food Science* 67, 1515-1521.

Kim, S. H., Price, R. J., Morrissey, M. T., Field, K. G., Wei, C. I. and An, H. J. 2002 Histamine production by *Morganella morganii* in mackerel, albacore, mahi-mahi, and salmon at various storage temperatures. *Journal of Food Science* 67, 1522-1528.

Kim, S.-H., Wei, C., Clemens, R. A. and An, H. 2004 Review: Histamine accumulation in seafoods and its control to prevent outbreaks of scombroid poisoning. *Journal of Aquatic Food Product Technlogy* 13, 81-100.

Kimata, M. 1961 "The histamine problem" in *Fish as Food*, G. Borgstrøm, ed., Academic Press, New York, pp. 329-352.

Kjølby, A. 2005 (Personal Communication)

Klausen, N. K. and Huss, H. H. 1987b Growth and histamine production by *Morganella morganii* under various temperature conditions. *International Journal of Food Microbiology* 5, 147-156.

Klausen, N. K. and Huss, H. H. 1987a A rapid method for detection of histamine-producing bacteria. *International Journal of Food Microbiology* 5, 137-146.

Kobayashi, T., Kimura, B. and Fujii, T. 2000 Differentiation of *Tetragenococcus* populations occurring in products and manufacturing processes of puffer fish ovaries fermented with rice-bran. *International Journal of Food Microbiology* 56, 211-218.

Konagaya, Y., Kimura, B., Ishida, M. and Fujii, T. 2002 Purification and properties of a histidine decarboxylase from *Tetragenococcus muriaticus*, a halophilic lactic acid bacterium. *Letters in Applied Microbiology* **92**, 1136-1142.

Kose, S. and Erdem, M. E. 2004 An investigation of quality changes in anchovy (*Engraulis encrasicolus*, L. 1758) stored at different temperatures. *Turkish Journal of Veterinary & Animal Sciences* 28, 575-582.

Koutsoumanis, K. P., Taoukis, P. S., Drosinos, E. H. and Nychas, G.-J. E. 2000 Application of an Arrhenius model for the combined effect of temperature and CO_2 packaging on the spoilage microflora of fish. *Applied and Environmental Microbiology* 66, 3528-3534.

Kow-Tong, C. and Malison, M. D. 1987 Outbreak of scombroid fish poisoning, Taiwan. *American Journal of Public Health* 77, 1335-1336.

Kristinsson, H. G., Danyali, N. and Ua-Angkoon, S. 2007 Effect of filtered wood smoke treatment on chemical and microbial changes in mahi-mahi fillets. *Journal of Food Science* 72, C016-C024.

Laguerre, O., Derens, E. and Palagos, B. 2002 Study of domestic refrigerator temperature and analysis of factors affecting temperature: a French survey. *International Journal of Refrigeration* 25, 653-659.

Landete, J. M., Pardo, I. and Ferrer, S. 2006 Histamine, histidine, and growth-phase mediated regulation of the histidine decarboxylase gene in lactic acid bacteria isolated from wine. *FEMS Microbiology Letters* 260, 84-90.

Landete, J. M., de las Rivas, B., Marcobal, A. and Munoz, R. 2007 Molecular methods for the detection of biogenic amine-producing bacteria on foods. *International Journal of Food Microbiology* 117, 258-269.

Laurenti, G. 2004 Fish and Fishery Products World Apparent Consumption Stastistics Based on Food Balance Sheets. Food and Agricultural Organization of the United Nations (FAO), Rome.

Le Marc, Y., Huchet, V., Bourgeois, C. M., Guyonnet, J. P., Mafart, P. and Thuault, D. 2002 Modelling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. *International Journal of Food Microbiology* 73, 219-237.

Leask, A., Yankos, P. and Ferson, M. J. 2004 Fish, so foul! Foodborne illness caused by combined fish histamine and wax ester poisoning. *Communicable Diseases Intelligence* 28, 83-85.

Legroux, R., Levaditi, J. C., Boudin, G. and Bovet, D. 1946 Intoxications histaminiques collectives - consecutives a lingestion de thon frais. *Presse Medicale* 54, 545-546.

Lehane, L. and Olley, J. 2000 Review: Histamine fish poisoning revisited. *International Journal of Food Microbiology* 58, 1-37.

Leitão, M. F. F., Baldini, V. L. S., Uboldi Eiroa, M. N. and Destro, M. T. 1983 Bactèrias produtoras de histamina em pescado de origem marinha. *Coletanea- Instituto de Technologia de Almintos* 13, 83-98.

Lerke, P. A., Werner, S. B., Taylor, S. L. and Guthertz, L. S. 1978 Scombroid poisoning - report of an outbreak. *Western Journal of Medicine* 129, 381-386.

Leroi, F., Joffraud, J.-J., Chevalier, F. and Cardinal, M. 1998 Study of the microbial ecology of cold-smoked salmon during storage at 8°C. *International Journal of Food Microbiology* 39, 111-121.

López-Gálvez, D. E., de la Hoz, L. and Ordóñez, J. A. 1995 Effect of carbon dioxide and oxygen enriched atmospheres on microbiological and chemical changes in refrigerated tuna (*Thunnus alalunga*) steaks. *Journal of Agricultural and Food Chemistry* 43, 483-490.

López-Sabater, E. I., Rodríguez-Jerez, J. J., Hernández-Herrero, M. M. and Mora-Ventura, M. T. 1996a Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *International Journal of Food Microbiology* 28, 411-418.

López-Sabater, E. I., Rodríguez-Jerez, J. J., Hernández-Herrero, M. M. and Mora-Ventura, M. T. 1994a Evaluation of histidine decarboxylase activity of bacteria isolated from sardine (*Sardina pilchardus*) by an enzymic method. *Letters in Applied Microbiology* 19, 70-75.

López-Sabater, E. I., Rodríguez-Jerez, J. J., Hernández-Herrero, M. M., Roig-Sagués, A. X. and Mora-Ventura, M. T. 1996b Sensory quality and histamine formation during controlled decomposition of tuna (*Thunnus thynnus*). *Journal of Food Protection* 59, 167-174.

López-Sabater, E. I., Rodríguez-Jerez, J. J., Roig-Sagués, A. X. and Mora-Ventura, M. T. 1994b Bacteriological quality of tuna fish (*Thunnus thynnus*) destined for canning: Effect of tuna handling on presence of histidine decarboxylase bacteria and histamine level. *Journal of Food Protection* 57, 318-323.

Lorca, T. A., Gingerich, T. M., Pierson, M. D., Flick, G. J., Hackney, C. R. and Sumner, S. S. 2001 Growth and histamine formation of *Morganella morganii* in determining the safety and quality of inoculated and uninoculated bluefish (*Pomatomus saltatrix*). *Journal of Food Protection* 64, 2015-2019.

Lucas, P. M., Wolken, W. A. M., Claisse, O., Lolkema, J. S. and Lonvaud-Funel, A. 2005 Histamine-producing pathway encoded on an unstable plasmid in *Lactobacillus hilgardii* 0006. *Applied and Environmental Microbiology* 71, 1417-1424.

Luedeking, R. and Piret, E. L. 1959 A kinetic study of the lactic acid fermentation. Batch process at controlled pH. *J Biochem Microbiol Technol Eng* 1, 393-412.

Lyhs, U., Korkeala, H. and Bjorkroth, J. 2002 Identification of lactic acid bacteria from spoiled, vacuum-packaged "gravad" rainbow trout using ribotyping. *International Journal of Food Microbiology* 72, 147-153.

Lyons, D. E., Beery, J. T., Lyons, S. A. and Taylor, S. L. 1983 Cadaverine and aminoguanidine potentiate the uptake of histamine *in vitro* in perfused intestinal segments of rats. *Toxicology and Applied Pharmacology* 70, 445-458.

Mackie, I. M. and Fernández-Salguero, J. 1977 Histidine metabolism in fish - Urocanic acid in mackerel (*Scomber scombrus*). *Journal of the Science of Food and Agriculture* 28, 935-940.

Maga, J. A. 1978 Amines in Foods. *Crc Critical Reviews in Food Science and Nutrition* 10, 373-403.

Maijala, R. L., Eerola, S. and Hirn, J. A. 1996 Biogeeniset amiinit II. *Suomen Eläinlääkärilehti* 102, 75-80.

Maire, R., Dreiding, K. and Wyss, P. A. 1992 Incidence and clinical findings of scombroid-fish poisoning. *Schweizerische Medizinische Wochenschrift* 122, 1933-1935.

Malle, P., Valle, M. and Bouquelet, S. 1996 Assay of biogenic amines involved in fish decomposition. *Journal of AOAC International* 79, 43-49.

Marcobal, A., de las Rivas, B. and Munoz, R. 2006 Methods for the detection of bacteria producing biogenic amines on foods: A survey. *Journal für Verbraucherschutz und Lebensmittelsicherheit* 1, 187-196.

Marklinder, I. M., Lindblad, M., Eriksson, L. M., Finnson, A. M. and Lindqvist, R. 2004 Home storage temperatures and consumer handling of refrigerated foods in Sweden. *Journal of Food Protection* 67, 2570-2577.

Masson, F., Talon, R. and Montel, M. C. 1996 Histamine and tyramine production by bacteria from meat products. *International Journal of Food Microbiology* 32, 199-207.

Mattick, K. L., Jorgensen, F., Legan, J. D., Cole, M. B., Porter, J., Lappin-Scott, H. M. and Humphrey, T. J. 2000 Survival and filamentation of *Salmonella enterica* serovar Enteritidis PT4 and S*almonella enterica* serovar Typhimurium DT104 at low water activity. *Applied and Environmental Microbiology* 66, 1274-1279.

Mavromatis, P. and Quantick, P. C. 2002a "Histamine Toxicity and Scombroid Fish Poisoning: a Review" in *Seafoods - Quality, Technology and Nutraceutical Applications*, C. Alasalvar & T. Taylor, eds., Springer-verlag Berlin Heidelberg New York, pp. 89-103.

Mavromatis, P. and Quantick, P. C. 2002b Modification of Niven's medium for the enumeration of histamine-forming bacteria and discussion of the parameters associated with its use. *Journal of Food Protection* 65, 546-551.

Mayer, K. and Pause, G. 1972 Biogene amine in sauerkraut. *Lebensmittel-Wissenschaft und-Technologie* 5, 108-109.

McCabe, B. J. 1986 Dietary tyramine and other pressor amines in MAOI regimes: A review. *Journal of the American Dietetic Association* 86, 1059-1064.

McDonald, K. and Sun, D. W. 1999 Predictive food microbiology for the meat industry: A review. *International Journal of Food Microbiology* 52, 1-27.

McKellar, R. C. 2004 "Primary models" in *Modelling Microbial Responses in Food*, R. C. McKellar & X. Lu, eds., CRC Press, Boca Raton, pp. 21-62.

McMeekin, T. A., Olley, J., Ross, T. and Ratkowsky, D. A. 1993 Predictive Microbiology: Theory and Application. Research Studies Press Ltd., Taunton, UK.

McMeekin, T. A. and Ross, T. 2002 Predictive microbiology: providing a knowledge-based framework for change management. *International Journal of Food Microbiology* 78, 133-153.

Mejlholm, O. and Dalgaard, P. 2007a Modeling and predicting the growth of lactid acid bacteria in lightly preserved seafood and their inhibiting effect on *Listeria monocytogenes*. *Journal of Food Protection* (*Accepted*).

Mejlholm, O. and Dalgaard, P. 2007b Modeling and predicting the growth boundary of *Listeria monocytogenes* in lightly preserved seafood. *Journal of Food Protection* 70, 70-84.

Mellefont, L. A., McMeekin, T. A. and Ross, T. 2003 The effect of abrupt osmotic shifts on the lag phase duration of foodborne bacteria. *International Journal of Food Microbiology* 83, 281-293.

Merson, M. H., Baine, W. B., Gangaros, E. J. and Swanson, R. C. 1974 Scombroid fish poisoning - outbreak traced to commercially canned tuna fish. *Journal of the American Medical Association* 228, 1268-1269.

Middlebrooks, B. L., Toom, P. M., Douglas, W. L., Harrison, R. E. and McDowell, S. 1988 Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomorus maculatus*). *Journal of Food Science* 53, 1024-1029.

Mietz, J. L. and Karmas, E. 1977 Chemical quality of canned tuna as determined by high-pressure liquid chromatography. *Journal of Food Science* 42, 155-158.

Miki, M., Ishikawa, T. and Okayama, H. 2005 An outbreak of histamine poisoning after ingestion of the ground saury paste in eight patients taking isoniazid in tuberculous ward. *Internal Medicine* 44, 1133-1136.

Molenaar, D., Bosscher, J. S., Tenbrink, B., Driessen, A. J. M. and Konings, W. N. 1993 Generation of a proton motive force by histidine decarboxylation and electrogenic histidine histamine antiport in *Lactobacillus buchneri*. *Journal of Bacteriology* 175, 2864-2870.

Monod, J. 1949 The growth of bacterial cultures. *Annual reviews of microbiology* 3, 371-394.

Moret, S. and Conte, L. S. 1996 High-performance liquid chromatographic evaluation of biogenic amines in foods - An analysis of different methods of sample preparation in relation to food characteristics. *Journal of Chromatography A* 729, 363-369.

Morii, H., Cann, D. C. and Taylor, L. Y. 1988 Histamine formation by luminous bacteria in mackerel stored at low temperatures. *Nippon Suisan Gakkaishi* 54, 299-305.

Morii, H., Cann, D. C., Taylor, L. Y. and Murray, C. K. 1986 Formation of histamine by luminous bacteria isolated from scombroid fish. *Bulletin of the Japanese Society of Scientific Fisheries* 52, 2135-2141.

Morii, H., Izumi, Y., Kasama, K. and Ishimoto, R. 1994 Factors influencing histamine formation by psychrotrophic luminous bacteria *Photobacterium phosphorerum*. *Nippon Suisan Gakkaishi* 60, 773-777.

Morii, H. and Kasama, K. 1995 Changes in the activity of two histidine decarboxylases from *Photobacterium phosphoreum* during growth under different oxygen-tensions. *Fisheries Science* 61, 845-851.

Morii, H. and Kasama, K. 2004 Activity of two histidine decarboxylases from *Photobacterium phosphoreum* at different temperatures, pHs, and NaCl concentrations. *Journal of Food Protection* 67, 1736-1742.

Morrow, J. D., Margolies, G. R., Rowland, J. and Roberts, L. J. 1991 Evidence that histamine is the causative toxin of scombroid- fish poisoning. *New England Journal of Medicine* 324, 716-720.

Motil, K. J. and Scrimshaw, N. S. 1979 The role of exogenous histamine in scombroid poisoning. *Toxicology Letters* 3, 219-223.

Mukhopadhyay, A., He, Z. L., Alm, E. J., Arkin, A. P., Baidoo, E. E., Borglin, S. C., Chen, W. Q., Hazen, T. C., He, Q., Holman, H. Y., Huang, K., Huang, R., Joyner, D. C., Katz, N., Keller, M., Oeller, P., Redding, A., Sun, J., Wall, J., Wei, J., Yang, Z. M., Yen, H. C., Zhou, J. Z. and Keasling, J. D. 2006 Salt stress in *Desulfovibrio vulgaris* Hildenborough: An integrated genomics approach. *Journal of Bacteriology* 188, 4068-4078.

Muller, G. J., Lamprecht, J. H., Barnes, J. M., Devilliers, R. V. P., Honeth, B. R. and Hoffman, B. A. 1992 Scombroid poisoning - case series of 10 incidents involving 22 patients. *South African Medical Journal* 81, 427-430.

Neumeyer, K., Ross, T. and McMeekin, T. A. 1997 Development of a predictive model to describe the effect of temperature and water activity on the growth of spoilage pseudomonads. *International Journal of Food Microbiology* 38, 45-54.

Niven, C. F., Jeffrey, M. B. and Corlett, D. A. Jr. 1981 Differential plating medium for quantitative detection of histamine-producing bacteria. *Applied and Environmental Microbiology* 321-322.

Nuessle, W. F., Norman, F. C. and Miller, H. E. 1965 Pickled herring and tranylcypromine reaction. *Journal of the American Medical Association* 192, 726-727.

Oka, S., Fukunaga, K., Ito, H. and Takama, K. 1993 Growth of histamine producing bacteria in fish fillets under modified atmospheres. *Bulletin of the faculty of Fisheries, Hokkaido University* 44, 46-54.

Okuzumi, M. and Awano, M. 1983 Seasonal variations in numbers of psychrophilic and halophilic histamine-forming bacteria (N-group bacteria) in seawater and on marine fishes. *Bulletin of the Japanese Society of Scientific Fisheries* 49, 1285-1291.

Okuzumi, M., Awano, M. and Ohki, Y. 1984a Effects of temperature, pH value and NaCl concentration on histamine formation of N-group bacteria (psychrophilic and halophilic histamine-forming bacteria). *Bulletin of the Japanese Society of Scientific Fisheries* 50, 1757-1762.

Okuzumi, M., Okuda, S. and Awano, M. 1981 Isolation of psychrophilic and halophilic histamine-forming bacteria from *Scomber japonicus*. *Bulletin of the Japanese Society of Scientific Fisheries* 47, 1591-1598.

Okuzumi, M., Okuda, S. and Awano, M. 1982 Occurrence of psychrophilic and halophilic histamine-forming bacteria (N-Group bacteria) on/in red meat fish. *Bulletin of the Japanese Society of Scientific Fisheries* 48, 799-804.

Okuzumi, M., Yamanaka, H. and Kubozuka, T. 1984b Occurrence of various histamine-forming bacteria on/in fresh fishes. *Bulletin of the Japanese Society of Scientific Fisheries* 50, 161-167.

Okuzumi, M., Yamanaka, H., Kubozuka, T., Ozaki, H. and Matsubara, K. 1984c Changes in numbers of histamine-forming bacteria on/in common mackerel stored at various temperatures. *Bulletin of the Japanese Society of Scientific Fisheries* 50, 653-657.

Olofsson, T. C., Ahrne, S. and Molin, G. 2007 The bacterial flora of vacuum-packed cold-smoked salmon stored at 7°C, identified by direct 16S rRNA gene analysis and pure culture technique. *Journal of Applied Microbiology* 103, 109-119.

Omura, Y., Price, R. J. and Olcott, H. S. 1978 Histamine-forming bacteria isolated from spoiled skipjack tuna and jack mackerel. *Journal of Food Science* 43, 1779-1781.

Onal, A. 2007 A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry* 103, 1475-1486.

Paludan-Muller, C., Dalgaard, P., Huss, H. H. and Gram, L. 1998 Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5°C. *International Journal of Food Microbiology* 39, 155-166.

Parsons, M. E. and Ganellin, C. R. 2006 Histamine and its receptors. *British Journal of Pharmacology* 147, S127-S135.

Petäjä, E., Eerola, S. H. and Petäjä, P. 2000 Biogenic amines in cold-smoked fish fermented with lactic acid bacteria. *European Food Research and Technology* 210, 280-285.

Ramesh, A., Ananthalakshmy, V. K. and Venugopalan, V. K. 1989 Histamine production in Indian oil sardine and Indian mackerel by luminous bacteria. *Mircen-Journal of Applied Microbiology and Biotechnology* 5, 105-107.

Ramesh, A. and Venugopal, V. 1986 Densities and characteristics of histamine-forming luminous bacteria of marine fish. *Food Microbiology* 3, 103-105.

Ratkowsky, D. A., Olley, J., McMeekin, T. A. and Ball, A. 1982 Relationship between temperature and growth-rate of bacterial cultures. *Journal of Bacteriology* 149, 1-5.

Rawles, D. D. and Flick, G. J. 1996 Biogenic amines in fish and shellfish. *Advances in Food and Nutrition Research* 39, 330-365.

Rice, S. L., Eitenmiller, R. R. and Koehler, P. E. 1976 Biologically active amines in food - Review. *Journal of Milk and Food Technology* 39, 353-358.

Rodríguez-Jerez, J. J., López-Sabater, E. I., Roig-Sagués, A. X. and Mora-Ventura, M. T. 1993 Evolution of histidine decarboxylase bacterial groups during the ripening of Spanish semipreserved anchovies. *Journal of Veterinary Medicine B* 40, 533-543.

Rodríguez-Jerez, J. J., Mora-Ventura, M. T., López-Sabater, E. I. and Hernández-Herrero, M. M. 1994 Histidine, lysine and ornithine decarboxylase bacteria in Spanish salted semi-preserved anchovies. *Journal of Food Protection* 57, 784-787-791.

Rodtong, S., Nawong, S. and Yongsawatdigul, J. 2005 Histamine accumulation and histamine-forming bacteria in Indian anchovy (*Stolephorus indicus*). *Food Microbiology* 22, 475-482.

Rogers, P. L. and Staruszkiewicz, W. F. 1997 Gas chromatographic method for putrescine and cadaverine in canned tuna and mahi-mahi and fluorometric method for histamine (minor modification of AOAC Official Method 977.13): Collaborative study. *Journal of AOAC International* 80, 591-602.

Rogers, P. L. and Staruszkiewicz, W. F. 2000 Histamine test kit comparison. *Journal of Aquatic Food Product Technology* 9, 5-17.

Ross, T. 1999 Predictive Microbiology for the Meat Industry. Meat and Livestock Australia. pp. 196.

Ross, T. and Dalgaard, P. 2004 "Secondary models" in *Modelling Microbial Responses in Food*, R. C. McKellar & X. Lu, eds., CRC Press, Boca Raton, pp. 63-150.

Rosso, L., Lobry, J. R., Bajard, S. and Flandrois, J. P. 1995 Convenient model to describe the combined effects of temperature and pH on microbial-growth. *Applied and Environmental Microbiology* 61, 610-616.

Russell, F. E. and Maretic, Z. 1986 Scombroid poisoning - minireview with case-histories. *Toxicon* 24, 967-973.

Ryser, E. T., Taylor, S. L. and Marth, E. H. 1984 Amine production during associative growth of gram-negative bacteria in tuna fish infusion broth. *Systematic and Applied Microbiology* 5, 545-554.

Sakabe, Y. 1973 Studies on allergylike food poisoning 2. studies on the relation between storage temperature and histamine production. *Journal of the Nara Medical Association* 24, 257-264.

Sanchez Guerrero, I. M., Vidal, J. B. and Escudero, A. I. 1997 Scombroid fish poisoning: A potentially life-threatening allergic-like reaction. *Journal of Allergy and Clinical Immunology* 100, 433-434.

Satter, J. and Lorenz, W. 1990 Intestinal diamine oxidases and enteral-induced histaminosis: studies on three prognostic variables in an epidemiological model. *Journal of Neural Transmission [Supplementum]* 32, 291-314.

Schachner, C. and Fodor, T. 1968 Scombroid poisoning. *Morbidity and Mortality Weekly Report* 17, 452.

Scoging, A. 1998 Scombrotoxic (histamine) fish poisoning in the United Kingdom: 1987 to 1996. *Communicable Disease and Public Health* 1, 204-205.

Self, T. H., Chrisman, C. R., Baciewicz, A. M. and Bronze, M. S. 1999 Isoniazid drugs and food interactions. *American Journal of the Medical Scinces* 317, 304-311.

Sergelidis, D., Abrahim, A., Sarimvei, A., Panoulis, C., Karaioannoglou, P. and Genigeorgis, C. 1997 Temperature distribution and prevalence of *Listeria* spp. in domestic, retail and industrial refrigerators in Greece. *International Journal of Food Microbiology* 34, 171-177.

Shakila, R. J., Vasundhara, T. S. and Rao, D. V. 1996 Inhibitory effect of spices on in vitro histamine production and histidine decarboxylase activity of *Morganella morganii* and on the biogenic amine formation in mackerel stored at 30°C. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* 203, 71-76.

Shakila, R. J., Vijayalakshmi, K. and Jeyasekaran, G. 2003 Changes in histamine and volatile amines in six commercially important species of fish of the Thoothukkudi coast of Tamil Nadu, India stored at ambient temperature. *Food Chemistry* 82, 347-352.

Shalaby, A. R. 1996 Significance of biogenic amines to food safety and human health. *Food Research International* 29, 675-690.

Shibatani, T., Nishimura, N., Nabe, K., Kakimoto, T. and Chibata, I. 1974 Enzymatic production of urocanic acid by *Achromobacter liquidum*. *Applied and Environmental Microbiology* 27, 688-694.

Shirai, T., Fuke, S., Yamaguchi, K. and Konosu, S. 1983 Studies on extractive components of salmonids .3. Amino-acids and related-compounds in the extracts of heated muscles of 4 species of salmon. *Bulletin of the Japanese Society of Scientific Fisheries* 49, 765-768.

Silla Santos, M. H. 1996 Biogenic amines: their importance in foods. *International Journal of Food Microbiology* 29, 213-231.

Silva, C. C. G., Da Ponte, D. J. B. and Enes Dapkevicius, M. L. N. 1998 Storage temperature effect on histamine formation in big eye tuna and skipjack. *Journal of Food Science* 63, 644-647.

Sims, G. G., Farn, G. and York, R. K. 1992 Quality indices for canned skipjack tuna: Correlation of sensory attributes with chemical indices. *Journal of Food Science* 57, 1112-1115.

Sjaastad, O. and Sjaastad, O. V. 1974 Catabolism of orally administrated ^{14}C-histamine in man. *Acta Pharmacologica et Toxocologica* 34, 33-45.

Skinner, G. E., Larkin, J. W. and Rhodehamel, E. J. 1994 Mathematical modeling of microbial growth: A review. *Journal of Food Safety* 14, 175-217.

Smart, D. R. 1992 Scombroid poisoning. A report of seven cases involving the Western Australian salmon, *Arripis truttaceus*. *The Medical Journal of Australia* 157, 748-751.

Smith, A. M., Hayden, M. A., Mc Cay, S. G., Zapatka, F. A. and Hamdy, M. K. 1982 Detection and confirmation of histamine-producing bacteria. *Bulletin of Environmental Contamination and Toxicology* 29, 618-623.

Smith, T. A. 1981 Amines in food. *Food Chemistry* 6, 169-200.

Smulevich, G., Droghetti, E., Focardi, C., Coletta, M., Ciaccio, C. and Nocentini, M. 2007 A rapid spectroscopic method to detect the fraudulent treatment of tuna fish with carbon monoxide. *Food Chemistry* 101, 1071-1077.

Stratton, J. E., Hutkins, R. W. and Taylor, S. L. 1991 Biogenic amines in cheese and other fermented foods: A review. *Journal of Food Protection* 54, 460-470.

Stratton, J. E. and Taylor, S. L. 1991 "Scombroid poisoning" in *Microbiology of Marine Food Products*, D. R. Ward & C. R. Hackney, eds., Van Nordstrand Reinhold, New York, pp. 331-350.

Su, S. C., Chou, S. S., Chang, P. C. and Hwang, D. F. 2000 Determination of biogenic amines in fish implicated in food poisoning by micellar electrokinetic capillary chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications* 749, 163-169.

Subburaj, M., Karunasagar, I. and Karunasagar, I. 1984 Incidence of histidine decarboxylating bacteria in fish and market environs. *Food Microbiology* 1, 263-267.

Sumner, S. S. and Taylor, S. L. 1989 Detection method for histamine-producing, dairy-related bacteria using diamine oxidase and leucocrystal violet. *Journal of Food Protection* 52, 105-108.

Suyama, M. and Yoshizaw, Y. 1973 Free amino-acid composition of skeletal-muscle of migratory fish. *Bulletin of the Japanese Society of Scientific Fisheries* 39, 1339-1343.

Tabor, C. W. and Tabor, H. 1985 Polyamines in microorganisms. *Microbiological Reviews* 49, 81-99.

Takahashi, H., Kimura, B., Yoshikawa, M. and Fujii, T. 2003 Cloning and sequencing of the histidine decarboxylase genes of Gram-negative, histamine-producing bacteria and their application in detection and identification of these organisms in fish. *Applied and Environmental Microbiology* 69, 2568.

Tanase, S., Guirard, B. M. and Snell, E. E. 1985 Purification and properties of a pyridoxal 5'-phosphate-dependent histidine-decarboxylase from *Morganella-Morganii*-AM-15. *Journal of Biological Chemistry* 260, 6738-6746.

Taylor, S. L. 1990 "Other microbial intoxications" in *Foorborne Diseases*, D. O. Cliver, ed., Academic Press, San Diego, pp. 159-170.

Taylor, S. L. 1986 Histamine food poisoning: toxicology and clinical aspects. *CRC Critical Reviews in Toxicology* 17, 91-128.

Taylor, S. L., Guthertz, L. S., Leatherwood, M., Tillman, F. and Lieber, E. R. 1978 Histamine production by food-borne bacterial species. *Journal of Food Safety* 173-187.

Taylor, S. L., Hui, J. Y. and Lyons, D. E. 1984 "Toxicology of scombroid poisoning" in *Seafood Toxins*, E. P. Ragelis, ed., American Chemical Society, Washington D.C., pp. 417-430.

Taylor, S. L., Keefe, T. J., Windham, E. S. and Howell, J. F. 1982 Outbreak of histamine poisoning associated with consumption of Swiss cheese. *Journal of Food Protection* 45, 455-457.

Taylor, S. L. and Lieber, E. R. 1979 In vitro inhibition of rat intestinal histamine-metabolozing enzymes. *Food and Cosmetics Texocology* 17, 237-240.

Taylor, S. L. and Speckhard, M. W. 1984 Inhibition of bacterial histamine production by sorbate and other antimicrobial agents. *Journal of Food Protection* 47, 508-511.

Taylor, S. L. and Speckhard, M. W. 1983 Isolation of histamine-producing bacteria from frozen tuna. *Marine Fisheries Review, National Oceanic and atmospheric Administration* 45, 4-6.

Taylor, S. L., Stratton, J. E. and Nordlee, J. A. 1989 Histamine poisoning (scombroid fish poisoning) - An allergy-like intoxication. *Journal of Toxicology-Clinical Toxicology* 27, 225-240.

Taylor, S. L. and Woychik, N. A. 1982 Simple medium for assessing quantitative production of histamine by Enterobacteriaceae. *Journal of Food Protection* 45, 747-751.

ten Brink, B., Damink, C., Joosten, H. M. L. J. and Huis in´t Veld, J. H. J. 1990 Occurrence and formation of biologically active amines in foods. *International Journal of Food Microbiology* 11, 73-84.

Todd, E. C. D. 1997 Seafood-associated diseases and control in Canada. *Revue Scientific et Technique Office International des Epizooties* 16, 661-672.

Tolmasky, M. E., Actis, L. A. and Crosa, J. H. 1995 A histidine-decarboxylase gene encoded by the *Vibrio anguillarum* plasmid Pjm1 is essential for virulence - Histamine is a precursor in the biosynthesis of anguibactin. *Molecular Microbiology* 15, 87-95.

Torres, S., Roeckel, M. and Marti, M. C. 2002 Histamine formation by *Morganella morganii* isolated from *Trachurus murphyii* (Chilean mackerel). *Latin American Applied Research* 32, 209-214.

Tsai, Y. H., Chang, S. C., Kung, H. F., Wei, C. I. and Hwang, D. F. 2005a Histamine production by *Enterobacter aerogenes* in sailfish and milkfish at various storage temperatures. *Journal of Food Protection* 68, 1690-1695.

Tsai, Y. H., Kung, H. D., Lee, T. M., Lin, G. T. and Hwang, D. F. 2004 Histamine-related hygienic qualities and bacteria found in popular commercial scombroid fish fillets in Taiwan. *Journal of Food Protection* 67, 407-412.

Tsai, Y. H., Kung, H. F., Lee, T. M., Chen, H. C., Chou, S. S., Wei, C. I. and Hwang, D. F. 2005b Determination of histamine in canned mackerel implicated in a food borne poisoning. *Food Control* 16, 579-585.

Turner, M. E., Blumenst, B. A. and Sebaugh, J. L. 1969 A generalization of logistic law of growth. *Biometrics* 25, 577-580.

Uragoda, C. G. and Kottegoda, S. R. 1977 Adverse reactions to isoniazid on ingestion of fish with a high histamine content. *Tubercle* 58, 83-89.

USDA 2005 Dietary Guidelines for Americans 2005, U.S. Department of Health and Human Services, U.S. Department of Agriculture.

Vaaler, G. L., Brasch, M. A. and Snell, E. E. 1986 Pyridoxal 5'-phosphate-dependent histidine-decarboxylase - nucleotide-sequence of the hdc gene and the corresponding amino-acid-sequence. *Journal of Biological Chemistry* 261, 1010-1014.

Van Gelderen, C. E. M., Savelkoul, T. J. F., Van Ginkel, L. A. and Van Dokkum, W. 1992 The effects of histamine administered in fish samples to healthy-volunteers. *Journal of Toxicology-Clinical Toxicology* 30, 585-596.

Van Poelje, P. D. and Snell, E. E. 1990 Pyruvoyl-dependent enzymes. *Annual Review of Biochemistry* 59, 29-59.

Van Spreekens, K. J. A. 1987 "Histamine production by the psychrophilic flora" in *Seafood Quality Determination*, D. E. Kramer & J. Liston, eds., Elsevier Science Publishers, London, pp. 309-318.

Veciana-Nogués, M. T., Bover-Cid, S., Mariné-Font, A. and Vidal-Carou, M. C. 2004 Biogenic amine production by *Morganella morganii* and *Klebsiella oxytoca* in tuna. *European Food Research and Technology* **218**, 284-288.

Veciana-Nogués, M. T., Mariné-Font, A. and Vidal-Carou, M. C. 1997 Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP-related compounds, volatile amines and organoleptic changes. *Journal of Agricultural and Food Chemistry* 45, 2036-2041.

Walker, S. E., Shulman, K. I., Tailor, S. A. N. and Gardner, D. 1996 Tyramine content of previously restricted foods in monoamine oxidase inhibitor diets. *Journal of Clinical Psychopharmacology* 16, 383-388.

Wauters, G., Avesani, V., Charlier, J., Janssens, M. and Delmee, M. 2004 Histidine decarboxylase in Enterobacteriaceae revisited. *Journal of Clinical Microbiology* 42, 5923-5924.

Welch, A. A., Lund, E., Amiano, P., Dorronsoro, M., Brustad, M., Kumle, M., Rodriguez, M., Lasheras, C., Janzon, L., Jansson, J., Luben, R., Spencer, E. A., Overvad, K., Tjonneland, A., Clavel-Chapelon, F., Linseisen, J., Klipstein-Grobusch, K., Benetou, V., Zavitsanos, X., Tumino, R., Galasso, R., Bueno-de-Mesquita, H. B., Ocke, M. C., Charrondiere, U. R. and Slimani, N. 2002 Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutrition* 5, 1273-1285.

Wendakoon, C. N. and Sakaguchi, M. 1992 Nonvolatile amine production in mackerel muscle during growth of different bacterial species. *Journal of the Food Hygienic Society of Japan* 33, 39-45.

Wendakoon, C. N. and Sakaguchi, M. 1993 Combined effect of sodium-chloride and clove on growth and biogenic-amine formation of *Enterobacter aerogenes* in mackerel muscle extract. *Journal of Food Protection* 56, 410-413.

Whiting, R. C. 1995 Microbial modeling in foods. *Critical Reviews in Food Science and Nutrition* 35, 467-494.

Wilson, J. F. 2004 Balancing the risks and benefits of fish consumption. *Annals of Internal Medicine* 141, 977-980.

Wu, M. L., Yang, C. C., Yang, G. Y., Ger, J. and Deng, J. F. 1997 Scombroid fish poisoning: An overlooked marine food poisoning. *Veterinary and Human Toxicology* 39, 236-241.

Wu, S. F. and Chen, W. 2003 An outbreak of scombroid fish poisoning in a kindergarten. *Acta paediatrica Taiwan* 44, 297-299.

Yamamoto, Y., Nakahara, F., Hashiguchi, R. and Kushima, H. 1991 Distribution of *Morganella morganii* in raw fish products on the market and the effects of temperature and concentration of sodium chloride on histamine formation by *Morganella morganii*. *Japanese Journal of Food Microbiology* 7, 159-165.

Yamanaka, H. 1989 Changes in polyamines and amino-acids in scallop adductor muscle during storage. *Journal of Food Science* 54, 1133-1135.

Yamanaka, H., Itagaki, K., Shiomi, K., Kikuchi, T. and Okuzumi, M. 1985 Influences of the concentration of sodium chloride on the formation of histamine in the meat of mackerel. *Journal of the Tokyo University of Fisheries* 72, 51-56.

Yamanaka, H., Shimakura, K., Shiomi, K., Kikuchi, T. and Okuzumi, M. 1987 Occurrence of allergy-like food poisoning caused by mirin - Seasoned meat of dorado (*Coryphaena hippurus*). *Journal of the Food Hygienic Society of Japan* 28, 354-358.

Yamanaka, H., Shiomi, K. and Kikuchi, T. 1989 Cadaverine as a potential index for decomposition of salmonid fishes. *Journal of the Food Hygienic Society of Japan* 30, 170-174.

Yoshinaga, D. H. and Frank, H. A. 1982 Histamine-producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamis*). *Applied and Environmental Microbiology* 44, 447-452.

Zwietering, M. H., Wijtzes, T., De Wit, J. C. and Van't Riet, K. 1992 A decision support system for prediction of the microbial spoilage in foods. *Journal of Food Protection* 55, 973-979.

Paper 1

Emborg, J., Laursen, B. G. and Dalgaard, P.

Significant histamine formation in tuna (*Thunnus albacares*) at 2°C - effect of vacuum- and modified atmosphere-packaging on psychrotolerant bacteria.

International Journal of Food Microbiology, 2005. vol. 101, 263-279.

The following parts of this publication results directly from the present Ph.D. project: (i) Investigation of an outbreak of histamine fish poisoning caused by tuna. This included isolation, identification and screening for histamine production of the dominating microflora. (ii) Challengetests, where the effect of an oxygen containing modified atmosphere on histamine formation was studied as a function of the initial bacterial concentration and different profiles of storage temperatures. Part of this paper, namely storage trial 1 and 2 as well as the identification of the microbiota of yellowfin tuna at the time of processing, has previously been handed in as a part of the Master thesis: “Histamine producing bacteria in tuna from Sri Lanka” by Birgit Groth Laursen and Jette Emborg, Danish Institute for Fisheries Research, DTU, 2003. Storage trial 3 originates from the Bachelor thesis of Jette Emborg and Birgit Groth Laursen: “Importance and formation of biogenic amines in fresh and thawed MAP fish” (in Danish), Danish Institute for Fisheries Research, DTU, 2001.

Paper 2

Emborg, J. and Dalgaard, P.

Formation of histamine and biogenic amines in cold-smoked tuna: An investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning.

Journal of Food Protection, 2006. vol. 69, 897-906.

Paper 3

Emborg, J., Dalgaard, P. and Ahrens, P.

Morganella psychrotolerans sp. nov., a histamine-producing bacterium isolated from various seafoods.

International journal of Systematic and Evolutionary Microbiology, 2006. vol. 56, 2473-2479.

Paper 4

Emborg, J. and Dalgaard, P.

Modelling and predicting the growth and histamine formation by *Morganella psychrotolerans*.

(In preparation)

Paper 5

Dalgaard, P., Emborg, J., Kjølby, A., Sørensen, N. D., and Ballin, N. Z.

Histamine and biogenic amines – formation and importance in seafood
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